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Jardiance® (empagliflozin) comprimate filmate 10 mg, 25 mg. Indicaţii terapeutice: Tratamentul adulților cu diabet zaharat tip 2 insuficient controlat, ca adjuvant la dietă și exercițiul fizic: ca monoterapie atunci când metformina este considerată inadecvată din cauza intoleranței; în plus față de alte medicamente pentru tratamentul diabetului zaharat. **Doze şi mod de administrare:** Doza inițială este de 10 mg o dată pe zi (în monoterapie și în terapie asociată suplimentară cu alte medicamente pentru tratamentul diabetului zaharat). La pacienții care tolerează empagliflozin 10 mg o dată pe zi, care prezintă RFGe ≥60 ml/min/1,73 m^2 și care necesită un control glicemic mai strict, doza poate fi crescută la 25 mg o dată pe zi. Doza zilnică maximă este de 25 mg. Când empagliflozin este utilizat în asociere cu o sulfoniluree sau cu insulină, se poate avea în vedere o doză mai mică de sulfoniluree sau de insulină, pentru a reduce riscul de hipoglicemie. *IR* Nu este necesară ajustarea dozei la pacienți cu RFGe ≥60 ml/min/1,73 m^2 sau ClCr ≥60 ml/min. Administrarea nu trebuie inițiată la pacienți cu RFGe <60 ml/min/1,73 m^2 sau ClCr <60 ml/min. La pacienții la care valorile RFGe scad în mod persistent sub 60 ml/min/1,73 m^2 sau ClCr sub 60 ml/min, doza trebuie ajustată sau menținută la 10 mg o dată pe zi. Administrarea trebuie întreruptă la pacienții cu valori ale RFGe aflate persistent sub 45 ml/min/1,73 m^2 sau cu valori ale ClCr aflate persistent sub 45 ml/min. Nu trebuie utilizat la pacienții cu BRST sau cărora li se efectuează dializă. *IH* Nu este necesară ajustarea dozei la pacienți cu IH. Nu se recomandă utilizarea la pacienți cu IH severă. *Vârstnici* La pacienții cu vârsta de 75 ani și peste, trebuie avut în vedere un risc crescut de depleție volemică. Nu se recomandă începerea tratamentului cu empagliflozin la pacienții cu vârsta de 85 ani și peste. *Copii și adolescenți* La acest grup de pacienți, siguranța și eficacitatea administrării empagliflozin nu au fost încă stabilite. Nu sunt disponibile date. Mod de administrare Comprimatele pot fi administrate cu sau fără alimente, înghițite întregi cu apă. Dacă se omite o doză, aceasta trebuie administrată imediat ce pacientul își aduce aminte. În aceeași zi nu trebuie administrată o doză dublă. **Contraindicaţii:** Hipersensibilitate la substanţa activă sau la oricare dintre excipienţi. **Atenţionări şi precauţii speciale pentru utilizare:** Au fost raportate cazuri rare de CAD, inclusiv cazuri cu risc vital și cazuri letale la pacienți tratați cu inhibitori de SGLT2, inclusiv empagliflozin. Într-o serie de cazuri, natura afecțiunii a fost atipică, cu valori ale glicemiei mai mici de 14 mmol/l (250 mg/dl). Nu se cunoaște dacă CAD are o probabilitate mai mare de apariție în cazul utilizării dozelor mai mari de empagliflozin. Riscul de CAD trebuie luat în considerare în cazul apariției unor simptome nespecifice. Pacienții trebuie evaluați pentru depistarea CAD imediat ce apar aceste simptome, indiferent de valorile glicemiei. La pacienții unde se suspectează sau este diagnosticată CAD, tratamentul trebuie întrerupt imediat. Tratamentul trebuie întrerupt la pacienții care au fost spitalizați pentru intervenții chirurgicale majore sau afecțiuni medicale acute grave. La acești pacienți se recomandă monitorizarea cetonelor. Se preferă măsurarea valorii cetonelor din sânge, față de valoarea din urină. Tratamentul cu empagliflozin poate fi reînceput când valorile cetonelor s-au normalizat și starea pacientului a fost stabilizată. Înainte de a iniția tratamentul, trebuie luate în considerare antecedentele pacientului care pot predispune la CAD. Nu se recomandă reluarea tratamentului cu inhibitor de SGLT2 la pacienții cu antecedente de CAD dezvoltată în timpul tratamentului cu inhibitor de SGLT2 decât dacă a fost identificat și rezolvat un alt factor precipitant evident. Siguranța și eficacitatea empagliflozin la pacienții cu diabet de tip 1 nu a fost stabilită și empagliflozin nu trebuie utilizat pentru tratarea pacienților cu diabet de tip 1. *Monitorizarea funcției renale:* înainte de începerea tratamentului și periodic în timpul tratamentului, cel puțin anual; înainte de începerea tratamentului concomitent cu orice medicament care poate avea impact negativ asupra funcției renale. Leziuni hepatice Cazuri de leziuni hepatice au fost raportate atunci când s-a administrat empagliflozin în cadrul studiilor clinice. Nu s-a stabilit o relație de cauzalitate între empagliflozin și leziunea hepatică. Risc de depleție volemică Se impune prudență la pacienții la care scăderea tensiunii arteriale indusă de empagliflozin ar putea prezenta un risc. Până la corectarea pierderii de lichide, se va avea în vedere întreruperea temporară a tratamentului cu empagliflozin. Infecții ale căilor urinare În cadrul unor studii cumulate controlate cu placebo, frecvența infecțiilor căilor urinare raportate a fost similară la pacienții cărora li s-a administrat tratament cu empagliflozin 25 mg și placebo și mai mare la pacienții cărora li s-a administrat tratament cu empagliflozin 10 mg. La pacienții cărora li s-a administrat tratament cu empagliflozin s-au raportat ulterior punerii pe piață cazuri de infecții complicate ale căilor urinare. Întreruperea temporară a tratamentului cu empagliflozin trebuie avută în vedere la pacienții cu infecții complicate ale căilor urinare. Fasceită necrozantă care afectează perineul (gangrena Fournier). După punerea pe piață, s-au raportat cazuri de fasceită necrozantă care afectează perineul (afecțiune cunoscută și ca gangrena Fournier) la pacienți de ambele sexe tratați cu inhibitori de SGLT2. Este un eveniment rar, însă grav, care poate pune în pericol viața și care necesită intervenție chirurgicală urgentă și tratament cu antibiotice. În cazul în care există suspiciunea de gangrenă Fournier, se va întrerupe administrarea Jardiance® și se va institui imediat tratament (inclusiv antibiotice și debridare chirurgicală). IC Experiența la pacienți cu clasa NYHA **I-II** este limitată; nu există experiență în studiile clinice cu empagliflozin la pacienți cu clasa NYHA III-IV. În cadrul studiului EMPA-REG OUTCOME, la 10,1% dintre pacienți s-a raportat insuficiență cardiacă la momentul inițial. Scăderea numărului de decese de etiologie cardiovasculară la acești pacienți a fost similară cu cea înregistrată la populația de studiu totală. Evaluarea analizelor de laborator ale urinei Pacienții care urmează tratament cu Jardiance® vor avea glicozurie pozitivă. Lactoză Comprimatul conține lactoză. Pacienții cu afecțiuni ereditare rare de intoleranță la galactoză, deficit de lactază Lapp sau sindrom de malabsorbție a glucozei-galactozei nu trebuie să utilizeze acest medicament. **Fertilitatea, sarcina şi alăptarea:** Datele provenite din utilizarea empagliflozinului la gravide sunt inexistente. Este de preferat să se evite utilizarea Jardiance® în timpul sarcinii. Nu trebuie utilizat în timpul alăptării. Nu s-au efectuat studii privind efectele Jardiance® asupra fertilității la om. Studiile la animale nu au evidențiat efecte dăunătoare directe sau indirecte cu privire la fertilitate. **Efecte asupra capacităţii de a conduce vehicule şi de a folosi utilaje:** Jardiance® are influență mică asupra capacității de a conduce vehicule și de a folosi utilaje. Pacienții trebuie sfătuiți să ia măsuri de precauție pentru a evita hipoglicemia atunci când conduc vehicule și folosesc utilaje, în special atunci când Jardiance® este utilizat în asociere cu o sulfoniluree și/sau insulină. **Reacţii adverse (RA):** Siguranța administrării empagliflozinului a fost evaluată la un număr total de 15582 pacienți cu diabet zaharat de tip 2 incluși în studiile clinice. Reacția adversă raportată cel mai frecvent a fost hipoglicemia, atunci când se asociază cu sulfoniluree sau insulină. *Foarte frecvente:* hipoglicemie (atunci când se utilizează împreună cu sulfoniluree sau insulină). *RA frecvente:* candidoză vaginală, vulvovaginită, balanită și alte infecții genitale; infecții ale căilor urinare, sete, prurit, erupție cutanată tranzitorie, diureză crescută, creștere a lipidelor serice. *RA mai puțin frecvente:* urticarie, depleție volemică, disurie, creșterea creatininei sanguine/scăderea ratei de filtrare glomerulară, creșterea hematocritului. *RA rare:* CAD; *cu frecvență necunoscută :* angioedem, fasceită necrozantă care afectează perineul (gangrena Fournier). **Data ultimei reînnoiri a autorizației:** 14/02/2019. **Data revizuirii textului**: 10/2019. **Jardiance® se eliberează pe bază de prescripție medicală PRF. Pentru informații suplimentare vă rugăm să consultați RCP complet, disponibil la cerere.**

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Editorial

SARS-CoV-2 Epidemic and Diabetes

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An editorial related to the recent SARS-CoV-2 epidemics in a diabetes/metabolism journal might seem strange. However, the implications for diabetes subjects, usually defined as a vulnerable population in this respect [1], make worth this endeavor. Moreover, the SARS-CoV-2 epidemic comes on top of the long-time recognized "diabetes epidemic" that does not show signs of relenting. Thus, the latest International Diabetes Federation (IDF) data show that 463 million people are affected worldwide, an increase of 38 million compared to 2017 [2]. In addition, total healthcare costs associated with diabetes are estimated to around 760 billion USD [2].

In December 2019, the first cases of a new form of atypical pneumonia were reported in subjects visiting/associated with the Huanan Seafood Wholesale Market commercializing live animals. The epicenter of the outbreak was located in Wuhan, Hubei region, China [3]. Very soon, Chinese researchers identified a coronavirus from pneumonia patients as the pathogen agent and designated it as 2019-nCoV, better known as SARS-CoV-2 [4], while the disease induced by this virus was called COVID-19 (Corona Virus Disease – 2019) [5]. Already at the end of January 2020, the World Health Organization (WHO) declared COVID-19 a public health emergency, an epidemic in China, and a global pandemic in March 2020 [6]. Most recent estimates (but these figures will be widely surpassed at the time of publication of this material) indicate a total of almost 156,000 cases worldwide with 5,800 fatalities and approximately 75,000 recovered cases [7].

Coronaviruses affecting humans and other vertebrates for millennia are a group of single-stranded RNA viruses belonging to the family of Coronaviridae [8]. They are continually circulating in humans and are responsible for up to 30% of respiratory viral infections, usually mild respiratory diseases [9], but they can also induce digestive or neurologic diseases [8]. They became the focus of the scientific community in 2002 due to the epidemic of Severe Acute Respiratory Syndrome (SARS) induced by another member of the family – the SARSr-CoV virus and subsequently that of the Middle-East Respiratory Syndrome (MERS) induced by the MERSr-CoV [10], viruses with which SARS-CoV-2 share at least 70% genetic similarity.

Both SARSr-CoV and MERSr-CoV were transmitted from animals to humans and caused severe pulmonary syndromes in affected individuals. The main antigenic structure on the coronavirus envelope is a spike S protein [11]. This protein strongly interacts with the human Angiotensin-Converting Enzyme 2 (ACE2) that is expressed mainly in the lower respiratory tract and on alveolar epithelial cells. The attachment of the virus to the host cells depends on the binding of the S protein to the cell receptor, while the entry of the virus in the target cell is dependent on the S protein priming by cellular proteases, specifically the serine protease TMPRSS2 [9].

Estimates regarding the incubation period range from 1 to 14 days [12], with a mean of 5 days [13], but this is not yet fully elucidated. The most typical clinical signs include fever, sore throat, cough (productive or not), myalgia, and fatigue. When present, dyspnea is a sign of gravity. Upper respiratory tract signs such as runny nose and sneezing are less typical. Digestive symptoms (including diarrhea, vomiting, and abdominal pain) were also reported in up to 10% of patients, with the possibility of virus elimination in the stools and the fecal-oral transmission route [14]. However, the primary route of human-to-human transmission is

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via respiratory droplets and direct contact with surfaces contaminated with viral particles [10].

Preliminary data from the Chinese patients show that the vast majority of cases (~80%) are mild, 14% are severe (with pneumonia, respiratory distress, and hypoxemia), and 5% are critical (respiratory failure, multiple organ failure, septic shock) [5]. Severe and critical cases might have a low fever or even no fever. There are also cases with very mild symptoms and possibly asymptomatic cases [13] that could increase the probability of disease spreading in communities.

The main laboratory findings include a low lymphocyte count and increased C Reactive Protein (CRP) levels. Severe cases associate findings suggestive for organ damage (increased liver enzymes, muscle enzymes, creatinine, troponin, D-dimers, and others) [10]. Chest imagining in patients with pneumonia indicates small patchy infiltrates and interstitial changes in the initial stages and larger infiltrative shadows in both lungs in later stages [10]. Positive diagnosis in suspected patients is made by identifying viral RNA sequences using the real-time polymerase chain reaction (RT-PCR) technique or direct gene sequencing on biological samples, including nose/throat swabs, sputum, alveolar lavage or other samples [15, 16].

Currently, there is no specific antiviral treatment for SARS-CoV-2. General recommendations include supportive measures, prompt oxygen therapy by nasal catheter/mask at first signs of oxygen desaturation, antibiotic treatment only in cases of secondary bacterial supra-infection [10]. For severe cases, specific intensive care methods (including but not limited to invasive mechanical ventilation, circulation support, glucocorticoid use) should be promptly initiated [17].

Reported mortality rates vary widely. A very recent report in Lancet Infectious Diseases indicates that up to March 1st, 2020 mortality rate in China was 3.6% while outside China, the mortality rate was 1.5% [18].

Preventive measures to prevent the human spreading of the disease are of paramount importance. Preliminary data show that SARS-CoV-2 can be inactivated by heat, chloride disinfectants, 75% ethanol, and chloroform but not by chlorhexidine [10]. General recommendations include washing hands regularly and thoroughly, avoiding touching the face (specifically the eyes, nose, and mouth) before washing/disinfecting hands, cleaning and disinfecting objects/surfaces that are touched frequently, and trying to avoid close contact with subjects with symptoms of respiratory illness.

Moving to the issue of COVID-19 risk in diabetes subjects, current estimates indicate that diabetes is the second most common condition present in infected subjects. Thus, a report from Wuhan, China, indicates that 30% of COVID-19 patients had hypertension, and 19% had diabetes [19]. In addition, diabetes was associated with a death OR of 2.85 (95% CI 1.35 to 6.05) compared to non-diabetes subjects. Another recent report, also from Wuhan, indicated that 19% of patients who developed acute respiratory distress syndrome (ARDS) had diabetes (compared to only 5% of patients without ARDS) [20].

What can we do to improve the prognostic of diabetes subjects facing the SARS-CoV-2 epidemics? The recommendations for the general population should be strongly emphasized to our diabetes population. In addition, education instructions for sick days should be reminded and reinforced. Extra attention to good glycemic control could boost immunity and resistance to viral diseases. Proper hydration and insulin dose adjustments are essential in the presence of fever and flu-like symptoms. Rules for the proper treatment of hypoglycemic episodes should also be reminded. Besides, patients should be instructed to make a proper supply of diabetes medications in the case of quarantine that could last for several weeks. For people living alone, making sure that a relative or neighbor knows the patient has diabetes and could assist in case of an emergency is, of course, important. Finally, close contact with the diabetes healthcare team should be enforced.

Conflict of Interest

The author declares no conflict of interest.

References

1. Critchley JA, Carey IM, Harris T, DeWilde S, Hosking FJ, Cook DG. Glycemic control and risk of infections among people with type 1 or type 2 diabetes in a large primary care cohort study. Diabetes Care 41(10): 2127-2135, 2018.
2. International Diabetes Federation. IDF Diabetes Atlas – 9th Edition. Accessed at: http://www.diabetesatlas.org/ on March 11th, 2020.
3. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet 395(10223): 470-473, 2020.
4. Zhu N, Zhang D, Wang W et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 382(8): 727-733, 2020.
5. Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, Xia Z. Review of the clinical characteristics of Coronavirus Disease 2019 (COVID-19). J Gen Intern Med 2020 Mar 4. doi: 10.1007/s11606-020-05762-w.

https://doi.org/10.46389/rjd-2020-1001
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6. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. Accessed at: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 on March 13th 2020.
7. *** COVID-19 Coronavirus Outbreak. Accessed at: https://www.worldometers.info/coronavirus/ on March 15th, 2020.
8. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 17: 181–192, 2019.
9. Hoffmann M, Kleine-Weber H, Schroeder S et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020 Mar 4. pii: S0092-8674(20)30229-4. doi: 10.1016/j.cell.2020.02.052.
10. Deng SQ, Peng HJ. Characteristics of and public health responses to the coronavirus disease 2019 outbreak in China. J Clin Med 9(2): pii: E575, 2020.
11. Xu X, Chen P, Wang J et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci 63: 457-460, 2020.
12. Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395: 497–506, 2020.
13. Lauer SA, Grantz KH, Bi Q et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: Estimation and application. Ann Intern Med 2020.
14. Yeo C, Kaushal S, Yeo D. Enteric involvement of coronaviruses: is faecal-oral transmission of SARS-CoV-2 possible? Lancet Gastroenterol Hepatol 5(4): 335-337, 2020.
15. Corman VM, Landt O, Kaiser M et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 25(3): 2000044, 2020.
16. Wang W, Xu Y, Gao R et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA, 2020 [Epub ahead of print].
17. Murthy S, Gomersall CD, Fowler RA. Care for critically ill patients with COVID-19. JAMA, 2020.
18. Baud D, Qi X, Nielsen-Saines K, Musso D, Pomar L Favre G. Real estimates of mortality following COVID-19 infection Lancet Infectious Diseases, 2020.
19. Zhou F, Yu T, Du R et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet, 2020.
20. Wu C, Chen X, Cai Y et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med, 2020.

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Original Article

Significance of Antioxidants in Diabetic Individuals with Periodontitis

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Abstract

Introduction: The purpose of this research was to evaluate the serum level of vitamin C in patients with type 2 diabetes mellitus with and without periodontitis, and also to interpret whether raised or lessened serum vitamin C levels might be associated to a possibility for building oxidative stress in type 2 diabetes mellitus with periodontitis. **Material and Methods:** Investigation was conducted on diabetic subjects with and without periodontitis. The serum vitamin C level was determined using the 2, 4- dinitrophenylhydrazine method, and the spectrometer was used to read the concentration of serum vitamin C. The data attained were statistically calculated using the ANOVA test and post-hoc test. **Results:** The findings revealed that average serum vitamin C levels were reduced considerably among test groups (groups B, C, D) ($p< 0.0001$) in contrary to the healthy control group. Conclusion: The existing results are proof that reduced concentration of antioxidants such as vitamin C is related to a greater threat to the increase of oxidative stress in patients with type 2 diabetes mellitus and periodontitis.

Keywords: Antioxidants, Periodontitis, Oxidative stress, Type 2 diabetes mellitus, Vitamin C.

Introduction

Periodontal infection is one of the most common diseases that destroy the soft tissue, as well as the hard tissues surrounding the teeth. If such a condition is not managed, the bone supporting the teeth resorbs, leading to tooth loss [1, 2]. Hypothetically, individuals with periodontitis are at greater possibility of acquiring diabetes in addition to what is universally expected [3]. Diabetes is associated with compromised wound healing, exaggerated monocyte reaction to dental plaque microorganisms [4] in addition to decreased neutrophil chemotactic reactions, all of which can lead to increased local tissue damage. It has been assessed that diabetes mellitus affects over 552 million individuals globally by the year 2030 [5].

Vitamin C is an essential water-soluble micronutrient that has antioxidant, anti-carcinogenic, as well as anti-inflammatory properties [6-8]. Epidemiological reports state an adverse association among plasma vitamin C concentration as well as the severity of periodontitis [9, 10]. Vitamin C insufficiency leads to scurvy with decreased growth and collagen production, intensified periodontal destruction, bleeding, as well as tooth loss. On the other hand, Staudte et al. stated that the consumption of grapes leads to an upsurge in serum vitamin C concentration, and it lessens gingival bleeding [11]. It is very probable that these biochemical cycles of vitamin C [12, 13] result mainly from its chemical features of a reducing and chelating agent.

All these interpretations suggest that vitamin C is more closely associated with human pathophysiology than formerly believed. This study was done to assess the serum vitamin C concentration in patients with type 2 diabetes mellitus with and without periodontitis.

Material and Methods

Study Subjects and Ethical Approval

The study was conducted at the A. B. Shetty Institute of Dental Sciences in the outpatient department

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of periodontics located in Mangalore City, India. After obtaining ethical approval from the institutional ethics committee, a single-blinded study was conducted. The research comprised of a sum of 40 subjects in the age group 25 to 60 years. The individuals were separated into four categories, comprising of 10 members in every group as follows:

- Group A – Control healthy subjects
- Group B – Healthy subjects with periodontitis
- Group C- Subjects with type 2 diabetes mellitus and periodontitis
- Group D - Subjects with type 2 diabetes mellitus without periodontitis.

Selection criteria were established by inclusion and exclusion measures. The inclusion criteria were as follows: individuals diagnosed with diabetes taking oral anti-hyperglycemic agents according to the classification of the American Diabetes Association [14], systemically healthy individuals for group A, individuals diagnosed with chronic periodontitis, according to the 1999 classification of the American Academy of Periodontology for groups B and C [15]. The exclusion criteria involved pregnant or lactating women, smokers, individuals taking dietary supplements that underwent periodontal therapy and diabetic individuals with uncontrolled blood sugar levels

Clinical Assessment of Study Subjects

A regular proforma comprising the following information: name, age, gender, previous treatment record (if any), gingival score (Loe and Silness Gingival Index) [16], plaque score [16], clinical attachment and periodontal pockets according to the 1999 classification of chronic periodontitis [15] was recorded. Every individual was inspected by means of a mouth mirror, as well as Williams' graduated periodontal probe under artificial light. Written informed consent was obtained from each patient before the examination and drawing blood.

After the initial screening of all patients, the subjects were divided into groups, and 5 ml of venous blood was drawn to assess the Vitamin C level using the dinitrophenylhydrazine method, and the results were read using a visible spectrophotometer.

Analysis

The obtained results were tabulated and subjected to statistical analysis; the mean values and standard deviation were calculated. Tukey's post-hoc comparison test was carried out for comparison between the groups.

Results

The mean and standard deviation for serum Vitamin C is significantly higher in the control group in contrast to the test groups.

The ANOVA test showed significance in all parameters between groups and within groups, $p < 0.001$.

Discussion

Several human ailments are linked with a build-up of oxidative stress causing moreover from the distorted formation of free radicals or commencing the modified antioxidant elements or actions. Metal ions are recognized to perform an important part in living organisms, both in the development and physiological functions. Reduced absorption of trace components is witnessed in diabetic patients with periodontitis. Amongst the antioxidants usually eaten by humans, the antioxidant Vitamin C is of precise significance. Our study revealed a statistically significant negative correlation of Vitamin C among groups (B, C, D), as represented in Tables 1-3.

One chief association among periodontitis, as well as the chronic systemic inflammatory condition, is that they are all intensely related to the occurrence of oxidative stress. Oxidative stress reinforces the pathogenesis of periodontitis [17] and type 2 diabetes mellitus [18]. Oxidative stress may upshoot mitochondrial proton as well as electron seepage, which causes the main influence on mitochondrial linking inefficiency, leading to the formation of reactive oxygen species. Anoth-

Table 1: Mean and Standard Deviation for Serum Vitamin C.

Group	Vitamin C in mg/dl: -mean -std dev
Healthy	1.40 0.565
Healthy with Periodontitis	1.01 0.287
Type 2 Diabetes with Periodontitis	0.920 0.23
Type 2 Diabetes without Periodontitis	0.93 0.29

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Table 2: Quantitative analysis of Vitamin C was done using ANOVA.

Parameters	Groups	Sum of Square	Mean squares	F	P
Vitamin C in mg/dl	• Between groups • Within groups • Total	3.850 13.12 16.97	1.28 0.13	9.38	<0.001

Table 3: Post-Hoc test for Vitamin C.

Groups	Mean Difference	Std Error	P value
Healthy vs. Healthy with Periodontitis	0.389	0.104	0.002 <0.05
Healthy vs. Type 2 diabetes with Periodontitis	0.482	0.104	<0.001 significant
Healthy vs. Type 2 diabetes without periodontitis	0.465	0.104	<0.001 Significant
Healthy with periodontitis vs. Type 2 diabetes with periodontitis	0.093	0.104	0.806 i.e.>0.05
Healthy with periodontitis vs. type 2 diabetes without periodontitis	0.077	0.104	0.881 i.e.>0.05
Type 2 diabetes periodontitis vs. type 2 diabetes healthy	0.166	0.104	0.999 i.e.>0.05

er method by which dietary processed carbohydrates, as well as saturated fat consumption, produce free radicals is through to the role of advanced glycation end products (AGEs), and their receptor (RAGE), which plays a key role in the inflammatory actions leading to the development of diabetes in addition to its complications, or when minimally modified LDL activates Toll-like receptors, even reported of bounding to the C-reactive protein, which initiates metabolic disorders. Another reason of free radical production in humans is because of continuous production by antimicrobial peptides in the course of a degenerative action after a stimulus, for example, pathogens coated with opsonin, bacterial DNA or peptides, and stimulation of the hexose monophosphate shunt which makes use of molecular oxygen and NADPH as an electron donor [19].

Our results, as shown in Tables 1-3, are similar to previous studies where it was noticed that adults, as well as old aged individuals with type 2 diabetes mellitus, develop a drop in vitamin C levels, in addition to the upsurge of oxidative stress biomarkers [20] when matched to healthy subjects. Since Vitamin C derivatives aggregate in water solution, it has been suggested that it is guided and bounds at the outer layer of lipid bilayers tissues, thus leading to active retardation of free radicals occurring in the aqueous solution, and in effect, the restoration of fat-soluble antioxidants [21]. High blood sugar-induced oxidative stress plays an important part in the progress of diabetic complications, which is thought to be caused by increased reactive oxygen types production, in addition, due to reduced antioxidant protective action. An upsurge in blood glucose values in diabetic rats was detected after the initiation of diabetes by streptozotocin [22].

The association concerning periodontitis with vitamin C was also established by a British report [23], which revealed a converse association among plasma vitamin C concentration and serum antibody concentration of Porphyromonas gingivalis in an unmethodical subsample of the European population. Furthermore, in a non-scorbutic trial periodontitis rat model, it was revealed that vitamin C additions decreased the degenerative activity in the gingiva [24].

In a study conducted by Amaliya et al. that involved Indonesian inhabitants underprivileged to receive dental care, a noteworthy converse association among plasma vitamin C values and tissue degeneration was noted [25]. Similarly, a converse association between

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Vitamin C and periodontitis was found in the present study.

Vitamin C is a dominant antioxidant radical scrounger in the aqueous stage. Antioxidants help to soothe these extremely oversensitive free radicals, thus preserve the organizational as well as working integrity of cells. The relationship with periodontal-pathogenic microorganisms interrupts this delicate equilibrium, causing collagen breakdown and subsequent periodontal pocket development. An upsurge in the serum vitamin C concentration will have a beneficial result on periodontitis. As this vitamin is water-soluble, its oxidative counteraction activity depends mainly on the hydration status, identical to how leucocytes gather vitamin C to defend themselves against oxidative stress during phagocytosis.

Definite methods of conveyance, as well as digestion, assemble vitamin C in the tissues to improve its purpose as an enzyme cofactor and a foraging antioxidant [26]. Conversely, it is uncertain how vitamin C consumption disturbs gingival oxidative stress, gene expression encoding inflammation, as well as cell behavior in periodontal lesions, even though certain reports propose that vitamin C upsurges the number of collagen fibers in the renewing periodontal tissue in addition to clearing histamine in gingivitis [27]. Tests showed that the antioxidant action of Vitamin C includes the hydrogen atom transfer more than the transfer of electrons.

It was earlier projected that a continuous photochemical, as well as ambient production of active oxygen types, may compose a noteworthy threat in the etiology of periodontitis. On the other hand, under circumstances of raised radical formation as well as/or reduced antioxidant scavenging action, oxidative tissue injury may happen. In case of type 2 diabetes mellitus with periodontitis, reactive oxygen species formed by vitamin C might not only perform a part in the host protection contrary to the bacteria but also may affect oxidative injury to host tissues.

Conclusion

We assume that a drop in Vitamin C levels is linked to a more significant threat for type 2 diabetes mellitus with periodontitis when related to healthy individuals. Vitamin C acts as an effective scrounger of water-soluble free radicals as well as oxidants, thus guarding new biomolecules against oxidative destruction. More well-regulated clinical trials are essential to assess the result of reduced values of vitamin C on the threat of acquiring disease pathogenesis and the development of type 2 diabetes mellitus in patients with periodontitis, as well as before making general public health recommendations.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Chavarry NG, Vettore MV, Sansone C, Sheiham A. The relationship between diabetes mellitus and destructive periodontal disease: a meta-analysis. Oral Health Prev Den 7: 107-127, 2009.
2. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases, Lancet 366: 1809–1820, 2005 .
3. Preshaw PM, Alba AL, Herrera D, Jepsen S et al. Periodontitis and diabetes: a two-way relationship. Diabetologia 55(1): 21–31,2012.
4. Lakschevitz F, Aboodi G, Tenenbaum H, Glogauer M. Diabetes and periodontal diseases: interplay and links. Curr Diabetes Rev 7: 433-439, 2011.
5. Whiting DR, Guariguata L, Weil C, Shaw J. IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 94: 311-32, 2011.
6. Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. Proc Natl Acad Sci 85(24): 9748–9752, 1998.
7. Villacorta L, Azzi A, Zingg JM. Regulatory role of vitamins E and C on extracellular matrix components of the vascular system. Mol. Aspects Med 28(5-6): 507–537, 2007.
8. Padayatty SJ, Katz A, Wang Y et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr 22(1): 18–35, 2003.
9. Aurer-Kozelj J, Kralj-Klobucar N, Buzina R, Bacic M. The effect of ascorbic acid supplementation on periodontal tissue ultrastructure in subjects with progressive periodontitis. Int J Vitam Nutr Res 52(3): 333–341, 1982.
10. Amarasena N, Ogawa H, Yoshihara A, Hanada N et al. Serum vitamin C periodontal relationship in communitydwelling elderly Japanese . J Clin Periodontol 32(1): 3– 97, 2005.
11. Staudte H, Sigusch BW, Glockmann E. Grapefruit consumption improves vitamin C status in periodontitis patients. Br Dent J 199: 213–217, 2005.
12. Martell AE. Chelates of ascorbic acid. In: Ascorbic Acid: Chemistry, Metabolism, and Uses. (P.A. Seib and B.M. Tolbert, eds), Advances in Chemistry Series, 200, American Chemical Society, Washington DC, pp: 153-178, 1982.
13. Kroneck PMH, Armstrong FA, Merkle H, Marchesini A. Ascorbate oxidase: molecular properties and catalytic activity. In: Ascorbic Acid: Chemistry, Metabolism, and Uses. (P.A. Seib and

© 2020 The Authors

B.M. Tolbert, eds), Advances in Chemistry Series, 200, American Chemical Society, Washington DC, pp: 223-248, 1982.

14. ADA.I. Diagnosis and classification of Diabetes mellitus. Diabetic Care 35(1), 2102 .
15. Wiebe CB,Putnins EE. The periodontal disease classification system of the American academy of periodontology-An update . Journal of Canadian Dental Association 66(11): 594-597, 2000.
16. Loe H. The gingival index, the plaque index and the retention index system. Journal of Periodontology 38(6): 610-616, 1967.
17. Chapple ILC, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontology 2000 43: 160–232, 2007.
18. Allen EA, Matthews JB, O'Connor R, O'Halloran D, Chapple ILC. Periodontitis and type 2 diabetes: is oxidative stress a mechanistic link?. Scottish Medical Journal 54: 41– 47, 2009 .
19. Daniel MT, Abdelouahed K , Lise T, Tamas F. Effects of vitamin C supplementation on antioxidants and lipid peroxidation markers in elderly subjects with type 2 diabetes. Archives of Gerontology and Geriatrics 48: 67–72, 2009.
20. Stephen WS , Chian JJ, Mahmood M. Role of oxidative stress in diabetes-mediated vascular dysfunction: Unifying hypothesis of diabetes revisited. Vascular Pharmacology 57: 139–149, 2012.
21. Martin DR., Eleanor CK., John MW ,Michael JD. Oxidative damage to extracellular matrix and its role in human pathologies. Free Radical Biology & Medicine 44: 1973–2001, 2008.
22. Verma A, Dewangan P, Kesharwani D, Kela SP. Hypoglycemic and Hypolipidemic Activity of Scopoletin (Coumarin Derivative) in Streptozotocin Induced Diabetic Rats. Int J Pharm Sci Rev Res 22(1): 79-83, 2013.
23. Bergendi L, Benes L, Durackova Z, Ferencik M. Chemistry, physiology and pathology of free radicals. Life Sciences 65: 1865–1874, 1999.
24. Wilson JX. Regulation of vitamin C transport. Annual Review of Nutrition 25: 105–25, 2005.
25. Amaliya, Timmermann MF, Abbas F, Loos BG, Van der Weijden GA. Java project on periodontal diseases: the relationship between vitamin C and the severity of periodontitis. J Clin Periodontol 34(4): 299–304, 2007.
26. Nimbkar N, Lateef F. Treatment of essential hypertension and non-insulin dependent diabetes mellitus with vitamin C. Medical Hypotheses 68(5): 1126– 1133,2007.
27. Pussinen PJ, Laatikainen T, Alfthan G, Asikainen S, Jousilahti P. Periodontitis is associated with a low concentration of vitamin C in plasma. Cllin Dian Lab Immunol 10(5): 897–902, 2003.

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Original Article

The Effect of Protein Fractions of Avocado (*Persea americana*) on Biochemical Parameters in a Diabetic Rat Model

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Abstract

Introduction: The separation and inspection of the active protein constituents from the aqueous extract of avocado (*Persea americana*) by employing distinct biochemical ways is the primary target of this investigation. **Material and Methods:** Two compounds (A and B) were precipitated by ammonium sulfate and separated through gel filtration chromatography. **Results:** The glucose level decreased, compared to the oral administration because of the intraperitoneal administration of the protein fraction (B) and the concentrated aqueous extract. The molecular mass of the separated active protein fraction (peak B), was determined as 24,000 Daltons. Besides, certain blood components of alloxan-induced diabetic rats and normal rats were measured under the impact of the protein fraction B and the aqueous extract in doses of 50, 75, 100 and 125 mg per kilogram of body mass. The protein compound and the crude aqueous extract of the avocado with a dosage of 75 mg per kilogram of body weight significantly diminished the fasting blood sugar level in the normal rats compared to the normal set. Additionally, at the same dosage, a significant reduction in the serum total lipid and cholesterol levels was noticed. A noteworthy decline in the serum glucose, cholesterol, and total lipid levels in the diabetic rats, was beholden using the active protein in a dose of 75 mg/kg of body weight. **Conclusion:** This study showed that avocado (*Persea americana*) has the potential to be used for diabetes therapy due to active protein components.

Keywords: Avocado; *Persea Americana*; diabetes disease.

Introduction

Nearly 150 kinds of avocado can be found in tropical and subtropical regions, including *Persea Americana* [1]. In ethnomedicine, the seed of the avocado was utilized in varied applications, including treatment for dysentery, diarrhea, intestinal parasites, toothache, as well as skincare and in the beauty manufacture. Several health advantages, including weight loss and controlling human weight, have been exploited from the seed oil, in particular when applied by obese for weight loss [1-3]. A recent review reports the impact of avocado (*Persea americana*) on distinct elements of the metabolic syndrome, such as a combination of hazard parameters, like elevated cholesterol, blood sugar, body mass index, and blood pressure. All these parameters may cause a rise in the hazard of cardiovascular diseases and type 2 diabetes.

Avocados possess the most favorable impact on lipid profiles, with alterations of HDL-cholesterol, LDL-cholesterol, triglycerides, phospholipids, and total cholesterol. However, the seed, peel, leaves, and flesh of avocados have varied impacts on elements of the metabolic syndrome [4].

The primary target of this research was to study the impact of the protein constituents separated from the aqueous extract of avocado (*Persea americana*) on biochemical specifications in an experimental animal model with the finding of an active constituent, harboring insulin-like structure or action.

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Material and Methods

The crude aqueous extract preparation

Avocado (*Persea americana*) acquired from the local market in Iraq, weighing about 0.75 kg, cut in small sizes, combined with cold distilled water (DW) in a ratio of 1:3 w/v, and subsequently blended evenly for five minutes, using a mixer. The untreated, resulting material was mixed while on the ice, for another two hours, and subsequently kept in a refrigerator during the night.

Afterward, the blend was passing through some layers of filter to separate all remaining components. Ultimately, for isolating the supernatant, the mixture or the filtrate was then spun down at 8000 x g in a cold centrifuge for 15 minutes. Then, the supernatant was lyophilized in order to reduce the volume to about 1/3 for further use. The Lowry method was used to assess the total protein.

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Precipitation of the proteins

The ammonium sulfate precipitation was exploited to separate the active protein from the cold extract, and then, while stirring at 0°C, the addition of the cold crude aqueous extract was performed at a ratio of 75:100 w/v. The combination was kept in a fridge for 24 h, and then the mixture was spun down at 8000 x g for 15 minutes to separate the precipitated protein. Then, lyophilization was used to dry the precipitate. The sample was maintained at -20°C for further use.

The protein extract fractionation

About 5 ml of the concentrated sample was prepared by mixing 5 ml of distilled water with 150 mg of extract and spinning down. The gel-filtration chromatography was utilized for fractionating the protein ingredients from the plant, using a Sephadex G-75 (2.56 × 87 cm) column. The elution process was accomplished with DW.

Healthy adult rats, weighing between 150-170 g, were fasting for 16h and then randomly split into two principal sets. The first group was treated normally; meanwhile, in the second group, alloxan (125 mg/kg) was administered intra-peritoneally to induce diabetes. Each group was subsequently sub-separated into eight groups (each harboring four rats). The first set in each subgroup was served as a control set, while the rats in the other subgroups were administered intra-peritoneally the crude aqueous extract and the fractioned proteins (75 and 100 mg/kg). Following a 2-hr post-injection, and under ether anesthesia, blood samples were taken in non-heparinized micro-hematocrit capillary tubes by the orbital sinus rupture for subsequent analysis [5].

Assessment of cholesterol, total lipids, and glucose

Serum cholesterol and glucose levels were assessed based on the enzymatic techniques, using the Randox kit (UK) for glucose [6, 7]. The Chabral and Chardonnet techniques were applied for measuring the serum total lipid level [8].

Statistical analysis

Data analysis was performed using the statistical methods, including standard deviation, mean, minimum, and maximum. For comparison of diabetic and control rats, the student t-test was applied, and $P \leq 0.05$ was considered significant [9].

Results

Protein precipitation

The entire proteins from the crude aqueous extract were precipitated by the aid of the ammonium sulfate method [10]. About 54.19% of the precipitate content was protein, and the yield of the precipitation was 30.12%.

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Fractionation of total protein

Gel filtration chromatography was employed for the fractionation, using Sephadex G75, resulting in one considerable peak, by the elution volume of 411 ml (Figure 1).

The protein level per peak was quantitatively measured after gel filtration chromatography, resulting in 15.9% and 46.1% proteins in peak A and B, respectively.

A pre-calibrated column having known protein molecular weight (MW) was used after gel filtration chromatography to determine the MM of the protein, as demonstrated in Table 1. The MW of the protein in peak B and A was estimated at 23000 and 42000 D, as shown in Figure 2.

The effects of the isolated protein component and the crude aqueous extract on glucose, total lipids, and cholesterol after intraperitoneal administration in normal rats

The crude aqueous extract and associated protein impacts on total lipids, cholesterol, and glucose, in healthy rats are shown in Table 2.

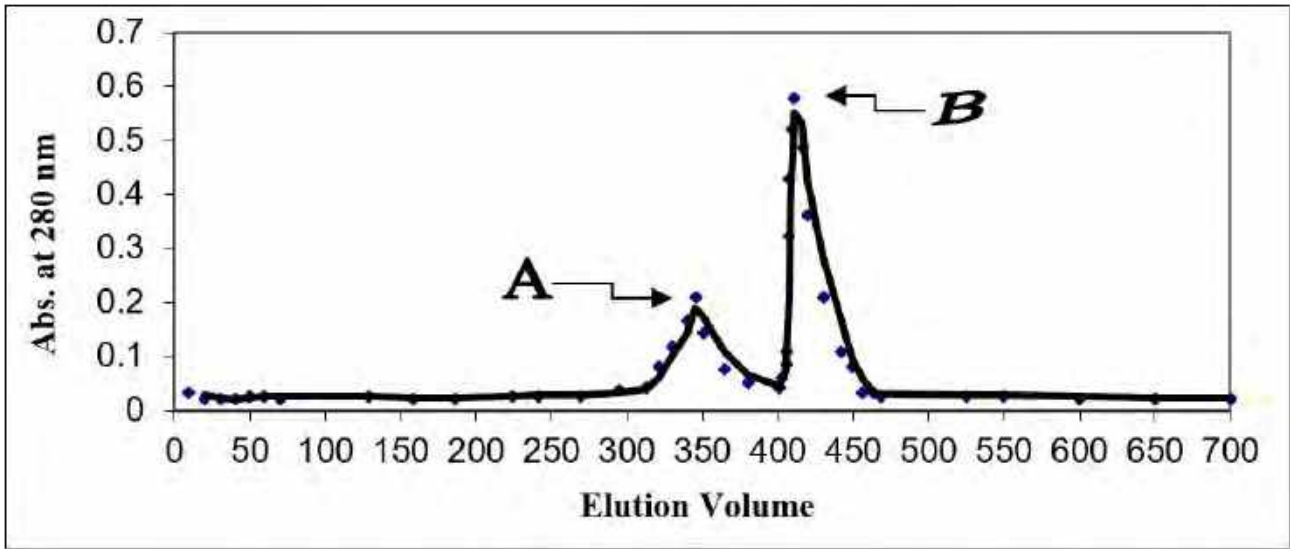

Figure 1: Elution profile of the total protein precipitate from avocado (Persea americana) - on a Sephadex G75 column (2.56 × 87cm); each fraction was eluted with 10 ml DW at a flow rate of 40 ml/h.

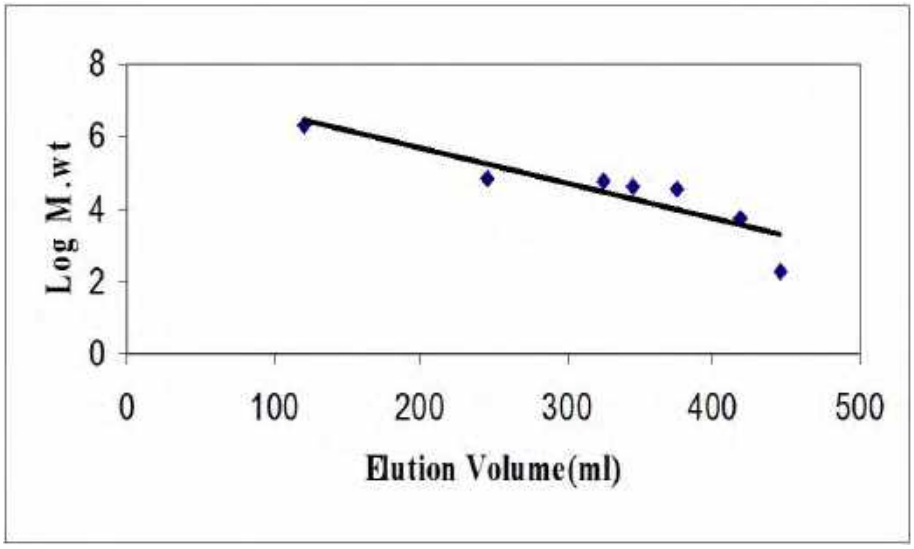

Figure 2: A logarithmic graph of elution volumes vs. MW of known proteins using a Sephadex G-75.

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Table 1: Molecular weights and the associated elution volumes of separated proteins on a Sephadex G 75.

Compounds	Molecular weight (Dalton)	Elution volume (ml)
Blue dextran	2000000	121
Bovine serum albumin (BSA)	67000	246
α- amylase	58000	324
Eggs albumin	45000	345
Pepsin	36000	375
Insulin hormone	5750	418
Tryptophan	204	446

Table 2: Intraperitoneal administration of the crude aqueous extract and associated isolated protein components and the influences on serum total lipids, cholesterol and glucose, in normal rats.

Groups	Glucose (mmol/L)	Cholesterol (mmol/L)	Total lipids (mg/dL)
Control	4.70 ± 0.435	2.90 ± 0.158	487.1 ± 11.762
Crude aqueous extract	3.50 ± 0.506*	2.44 ± 0.20	403.8 ± 99.97
peak A fraction at 125 mg/kg	3.94 ± 0.19	2.65 ± 0.10	413.5 ± 78.90
peak A fraction at 100 mg/kg	3.90 ± 0.25	2.50 ± 0.13	409.5 ± 78.78
peak A fraction at 75 mg/kg	3.76 ± 0.39	2.21 ± 0.21	393.9 ± 70.30
peak A fraction at 50 mg/kg	3.75 ± 0.32	2.16 ± 0.29	383.8 ±73.53
peak B fraction at 125 mg/kg	3.76 ± 0.53	2.61 ± 0.14	377.5 ± 51.77*
peak B fraction at 100 mg/kg	3.71 ± 0.54	2.58 ± 0.17	371.5 ± 31.66**
peak B fraction at 125 mg/kg	3.76 ± 0.53	2.61 ± 0.14	377.5 ± 51.77*
peak B fraction at 100 mg/kg	3.71 ± 0.54	2.58 ± 0.17	371.5 ± 31.66**

Note: * Significant difference at P<0.05; ** Significant difference at P<0.001 .

Table 3: Effects of oral administration of the isolated protein compound and the crude aqueous extract on the lipid profile and serum glucose in normal rats.

Groups	Glucose (mmol/L)	Cholesterol (mmol/L)	Total lipids (mg/dL)
Control	4.70 ± 0.435	2.90 ± 0.158	487.1 ± 11.76
Crude aqueous extract	4.25 ± 0.33*	2.53 ± 0.26	435 ± 51.5
Peak A fraction at 125 mg/kg	4.76 ± 0.07	2.84 ± 0.16	457 ± 63.10
Peak A fraction at 100 mg/kg	4.75 ± 0.21	2.82 ± 0.19	456 ± 68.00
Peak A fraction at 75 mg/kg	4.77 ± 0.74	2.78 ± 0.16	435 ± 78.12
Peak A fraction at 50 mg/kg	4.76 ± 0.77	2.71 ± 0.17	438 ± 79.17
Peak B fraction at 125 mg/kg	4.76 ± 0.90	2.71 ± 0.13	433.4 ± 76.10
Peak B fraction at 100 mg/kg	4.67 ± 0.86	2.78 ± 0.12	438.4 ± 71.33

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Peak B fraction at 75 mg/kg	4.74 ± 0.74	2.77 ± 0.26	391 ± 83.11*
Peak B fraction at 50 mg/kg	4.73 ± 0.86	2.73 ± 0.16	337 ± 75.19*

Note: * Significant difference at P<0.05; ** Significant difference at P<0.001.

Table 4: The influence of the isolated protein compound and the crude aqueous extract on serum total lipids, cholesterol, and glucose in diabetic rats.

Groups	Glucose (mmol/L)	Cholesterol (mmol/L)	Total lipids (mg/dL)
Control	29.6 ± 1.019	3.02 ± 0.680	657.15 ± 29.747
Crude aqueous	20.6 ± 3.65*	2.14 ± 0.43	478.51 ± 32.92*
Peak B fraction at 125 mg/kg	23.2 ± 4.13	2.37 ± 0.43	485.53 ± 15.62*
Peak B fraction at 100 mg/kg	20.5 ± 4.24	2.32 ± 0.72	487.34 ± 13.32*
Peak B fraction at 75 mg/kg	17.6 ± 8.10*	1.71 ± 0.37*	294.40 ± 32.38**
Peak B fraction at 50 mg/kg	25.6 ± 3.81	2.58 ± 0.81	473.40 ± 35.73*

Note: * Significant difference at P≤0.05; ** Significant difference at P<0.001.

After the administration of the protein fraction from peak A, no significant decline was beholden in the serum glucose in normal rats.

Oral administration of the crude aqueous extract, the isolated protein component and the effects on total lipids, cholesterol and glucose in normal rats

The data regarding the influence of protein components and the crude aqueous extract on total lipids, glucose, and cholesterol in normal rats are presented in Table 3. The data showed no significant decrease in fasting blood sugar (FBS) in normal rats due to the protein fraction (Peak A, peak B).

The intraperitoneal administration of the isolated protein compound and the crude aqueous extract, and the influences on lipid profile and glucose, in diabetic rats

The objective was to examine the impact of the protein compound of active peak and the crude aqueous extract of avocado (*Persea americana*) on serum cholesterol, total lipids, and glucose in alloxan-induced diabetic rats. Alloxan can induce diabetes by damaging the Langerhans cells, causing a reduction in the generation and secretion of insulin [4, 6]. The data regarding the impact of intraperitoneal administration on blood parameters in diabetic rats are presented in Table 4.

Discussions

Intraperitoneal administration of the crude aqueous extract and associated isolated protein components and the influences on serum total lipids, cholesterol and glucose, in normal rats

A precious decline in the FBS level was attained after intraperitoneal administration of the crude aqueous extract of avocado (*Persea americana*), compared with the control group.

A regular avocado-containing diet results in less insulin requirement. Also, when body mass index (BMI, a scale for obesity) elevates, the favorable impacts on insulin requirements, improve even further [4]. In overweight people, the ratio of C-peptide/insulin is also lower, showing that the clearance speed of insulin from the liver is increased. This is happening in addition to the insulin-lowering characteristics of avocado-containing meals, which leads to a decrease in blood sugar levels [10].

The blood level of C-peptide is an indicator of the insulin produced by the pancreas. The pancreas makes proinsulin, which is split into insulin and C-peptide and then secreted into the circulation. Therefore, a low level of C-peptide signifies that a lower amount of insulin has been produced in the blood. As a result, the level of total lipids and cholesterol decreased in the blood, in comparison with the control group. The low cho-

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lesterol levels are a result of less cholesterol accumulation in the body, and high disintegration and discharge through the feces [4, 5].

Our data have also indicated that the administration of the protein component of peak B of avocado (*Persea americana*) with a dosage of 75 mg/kg, has resulted in the maximum decline (64%) in the FBS level compared to the control set, as shown in Table 2. There is a chance that the decline might be the outcome of the insulin-like activity of the protein component of avocado (*Persea americana*) [4] or due to the insulin-like shape of the protein component that attaches to insulin receptors and declines the FBS level. The protein component of peak B with a dosage of 75 mg per kilogram of body weight, presented in the same table, demonstrated a precious drop in total lipids and cholesterol. This might be the consequence of the inhibition of cholesterol production or a rise in the percentage of cholesterol excretion from the body, and the insulin-like activity may aid in lowering the serum cholesterol level [5, 11].

Avocado is a good point of supply for minerals, carotenoids, phenols, fatty acids, and vitamins. The anti-hypertensive, lipid-lowering, anti-obesity, anti-diabetic, anti-atherosclerotic, anti-thrombotic, and cardioprotective effects of avocado have been shown in other studies [4, 12, 13].

Effects of oral administration of the isolated protein compound and the crude aqueous extract on lipid profile and serum glucose in normal rats

Table 3 reports that the administration of the protein component led to a high decrease in total lipids, glucose, and cholesterol in diabetic rats, as in the case of normal rats. Nonetheless, the peak B protein compound at a dosage of 75 mg/kg was more influential in decreasing the previously mentioned characteristics in diabetic rats, in comparison to the normal rats. This declined that the impact of this investigation could help reduce the risk of hyperinsulinemia (high insulin levels in the blood), a complication related to type 2 diabetes [14, 15]. Therefore, in the diet of patients with non-insulin-dependent diabetes mellitus, the complex digestible carbohydrates can be replaced by monounsaturated fatty acids like avocado, as the principal source, which leads to the significant improvement of the lipid profile, maintenance over a suitable glycemic control, and present a proper alternative in managing the disease [14].

Some studies reported the insulin-like action of protein compounds, simplifying the entrance of glucose inside the cells, and leading to an increase in the associated metabolism [16-18].

Following oral administration of the protein fractions (Peak A and B) and the crude aqueous extract of avocado (*Persea americana*), an increase in the serum glucose was found. This might be due to the constituents such as proteins, polysaccharides, amino acid, and fats in the crude aqueous extract. As glycogenolysis, glycolysis, and gluconeogenesis become active, these compounds, after metabolic reactions, increase serum glucose. An increase in the serum glucose was also found after oral administration of the protein fraction, Peak A and peak B; the proteins may be inactivated by the proteolytic enzymes or broken down by the gastric juice.

Taken all together, the intraperitoneal administration of the protein fraction and the plant extract have a significant hypoglycemic effect compared to the oral administration, and it is the most recommended path.

The influence of the isolated protein compound and the crude aqueous extract on serum total lipids, cholesterol, and glucose in diabetic rats

As presented in Table 4, the administration of the protein component led to a high decrease in total lipids, glucose, and cholesterol in diabetic rats, as in the case of normal rats. Nonetheless, the peak B protein compound with a dosage of 75 mg/kg was more influential in decreasing the above-mentioned characteristics in diabetic rats, in comparison to the normal rats. This lowering impact of this investigation could contribute to the reduction of the risk of hyperinsulinemia (high insulin in the blood), a complication related to type 2 diabetes [14, 15]. Therefore, in the diet of patients with non-insulin-dependent diabetes mellitus, the complex digestible carbohydrates can be replaced by monounsaturated fatty acids like avocado, as the principal source, which leads to the significant improvement of the lipid profile, maintenance over a suitable glycemic control, and present a proper alternative in managing the disease [14]. Some studies reported the insulin-like action of protein compound, simplifying the entrance of glucose inside the cells, and leading to an increase in the associated metabolism [16-18].

Conclusion

In terms of novelty, this study showed that avocado (*Persea americana*) has the potential to be used for di-

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abetes therapy due to active protein components. Furthermore, the glucose and lipid ratio was smaller after having an avocado, demonstrating high liver potency in insulin clearance. Avocado also has antioxidants, such as carotenoids and vitamin C, which might contribute to the insulin regulation, which is applicable in reducing the blood sugar and lipids.

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Conflict of Interest

The author declares no conflict of interest.

References

1. Lopez Ledesma R, Frati Munari AC, Hernandez Dominguez BC et al. Monounsaturated fatty acid (avocado) rich diet for mild hypercholesterolemia. Arch Med Res 27(4): 519-23, 1996.
2. Alhassan AJ, Atiku M, Sule MS, Wudil AM. Effects of aqueous avocado pear (Persea americana) seed extract on alloxan induced diabetes rats. Greener Journal of Medical Sciences 2(1): 005-011, 2012.
3. Roger CR. The nutritional incidence of flavonoids: some physiological and metabolic considerations. Experientia 44(9): 725-33, 1988.
4. Tabeshpour J, Razavi BM, Hosseinzadeh H. Effects of Avocado (Persea americana) on Metabolic Syndrome: A Comprehensive Systematic Review. Phytother Res 31(6): 819-837, 2017.
5. Aljawadi ZAM. Isolation and Studying the Effect of Protein Fractions of Red chili pepper (Capsicum annuum L.) on Some Biochemical Parameters in Experimental Diabetic Rats. International Journal of Enhanced Research in Science. Technology & Engineering 7(2): 51-56, 2018.
6. Passing H, Bablok A. New biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry. Part I. J Clin Chem Clin Biochem 21(11): 709-20, 1983.
7. Tietz NW. Text book of Clinical Chemistry. 3rd ed. 1999: C.A. Burtis, E.R. Ashwood, W.B. Saunders. pp: 809-856.
8. Aljawadi ZAM. Isolation and Studying the Effect of Protein Fractions of White Cabbage (Brassica oleracea var. capitata) on Some Biochemical Parameters in Experimental Diabetic Rats. Int Res J Med Sci1(11): 17-22, 2013.
9. Torrie J, Steel R. Principles and Procedures of Statistics a Biometrical Approach. 2nd ed. Singapore: McGraw-Hill Inc, 1986
10. Mohamed M, Abdel N, Rashad M. Hypoglycemic and Hypocholesterolemia Effects of Intragastric Administration of Dried Red Chili Pepper (Capsicum Annum) in Alloxan-Induced Diabetic Male Albino Rats Fed with High-Fat-Diet. Journal of Food and Nutrition Research 2(11): 850-856, 2014.
11. Esayas K, Shimelis A, Ashebir F, Negussie R, Berhanu T, Gulelat D. Proximate composition, mineral content and antinutritional factors of some capsicum (Capsicum annum) varieties grown in Ethiopia. Bulletin of the Chemical Society of Ethiopia 25(3), 2011.
12. Mohiuddin AK. Natural Foods and Indian herbs of cardiovascular interest. Pharmacy & Pharmacology International Journal 7(2), 2019.
13. Wang L, Bordi PL, Flemig JA, Hill AM, Kris-Etherton PM. Effect of a moderate fat diet with and without avocados on lipoprotein particle number, size and subclasses in overweight and obese adults: a randomized, controlled trial. J Am Heart Assoc 4(1): e001355, 2015.
14. Aslan M, Orhan N, Orhan DD, Ergun F. Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes. J Ethnopharmacol 128(2): 384-9, 2010.
15. Liang YT, Tian XY, Chen JN et al. Capsaicinoids lower plasma cholesterol and improve endothelial function in hamsters. Eur J Nutr 52(1): 379-88, 2013.
16. Lerman-Garber I, Ichazo-Cerro S, Zamora-Gonzales J, Cardoso-Saldana G, Posadas-Romero C. Effect of a high-monounsaturated fat diet enriched with avocado in NIDDM patients. Diabetes Care 17(4): 311-5, 1994.
17. Ozgur M, Ozcan T, Akpinar-Bayizit A, Yilmaz-Ersan L. Functional compounds and antioxidant properties of dried green and red peppers. African Journal of Agricultural Research 6(25): 5638-44 , 2011.
18. Chandini R, Binoy JP, Udayabhaskaran V, Ramamoorthy KP. A study of non-obese diabetes mellitus in adults in a tertiary care hospital in Kerala, India. International Journal of Diabetes in Developing Countries 33(2): 83-85, 2013.

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Original Article

The Effect of Brewed Robusta Coffee Leaves on Insulin Levels and HOMA-IR Index in Metabolic Syndrome Rats

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Abstract

Introduction: Central obesity and insulin resistance have been known as the main risk factors for metabolic syndrome. Robusta coffee leaves could be a functional food to prevent metabolic syndrome with phytochemical compounds. This study aimed to prove the effectiveness of the administration of brewed Robusta coffee leaves on insulin levels and HOMA-IR index in metabolic syndrome rats. **Material and Methods:** Male Wistar rats (6 weeks old, 150-200 g, n = 36) were divided into six groups (n = 6 rats/group): normal control group (K1), metabolic syndrome control group without treatment (K2), mangiferin treatment group (X1), brewed Robusta coffee leaves 0.09 g/200BW group (X2), brewed Robusta coffee leaves 0.18 g/200BW group (X3), brewed Robusta coffee leaves 0.36 g/200BW group (X4). Besides the K1 group, the other groups were given a high-fat-fructose diet. Each dose of coffee leaves was brewed with 3.6 mL of water at 70°C for 10 minutes. The intervention was given for 28 days. **Results:** There was a significant increase in insulin levels ($p<0.000$) in all groups and a significant decrease in the HOMA-IR index in treatment groups ($p<0.000$). **Conclusions:** The administration of brewed Robusta coffee leaves increased insulin levels, and decreased the HOMA-IR index in metabolic syndrome rats with the most effective dose being 0.36 g/200gBW.

Keywords: Metabolic syndrome, Robusta coffee leaves, insulin levels, HOMA-IR index.

Introduction

Metabolic syndrome is characterized by a constellation of metabolic abnormalities, which increases the risk of type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CD) [1, 2]. Based on the third report of the National Cholesterol Education Program (NCEP ATP III), an individual has metabolic syndrome if three or more risk factors are present, including waist circumstance >102 cm in men, >88 cm in women; fasting blood glucose ≥110 mg/dl; triglycerides ≥150 mg/dl; high-density lipoprotein (HDL) <40 mg/dl in men and <50 mg/dl in women; blood pressure ≥130/85 mmHg [3]. Central obesity and insulin resistance have been known as the main risk factors for metabolic syndrome [1,4].

Obesity is related to insulin resistance; there is increased production of non-esterified fatty acids (NEFAs), glycerol, leptin, adiponectin, and cytokine proinflammatory in individuals with obesity. Elevated those products lead to insulin resistance [5]. Increased leptin levels affect insulin sensitivity, induce insulin resistance, and lipid accumulation [6]. Insulin resistance causes endothelial dysfunction and changes insulin-signaling pathways in muscle and adipose tissue and endothelial cells [7]. Obesity and insulin resistance have a similar key mechanism, which is oxidative stress [4, 6]. Oxidative stress occurs when excessive endogenous oxidative species leads to cell damage. Oxidative stress damages insulin secretion by pancreatic β cells and glucose transport in muscles and adipose tissue [6].

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Reactive oxygen species (ROS) or prooxidants, such as superoxide, hydrogen peroxide, and hydroxyl radical are oxidative stress agents [4]. In normal conditions, the production of ROS is balanced with antioxidants in the body, and if there is an imbalance between prooxidants and antioxidants, oxidative stress will occur [8].

Antioxidants are molecules that are stable enough to donate one electron to free radicals and neutralize them. Antioxidants inhibit cell damage, mainly through free radical scavengers [9]. The body needs endogenous defense mechanisms against free radicals; superoxide dismutase, catalase, and glutathione peroxide are endogenous antioxidants enzymes [10]. Besides the endogenous antioxidant defense system, consuming foods that contain antioxidants is recommended, such as natural sources that contain vitamins, flavonoids, and some other minerals [10, 11].

Coffee is a major beverage that has been consumed by people for a long time [12]. In most of the places in Indonesia, such as West Sumatera, people not only consume coffee from its seeds; however, they also consume its leaves, known as Kawa Daun or Kopi Kawa [13]. The most cultivated coffee plant in Indonesia is Robusta coffee (Coffea canephora) [14]. Coffee leaves contain many different phytochemicals that have health benefits that include anti-inflammatory, antioxidant, anti-diabetic, and controlling oxidative stress [15]. As shown by previous studies, the administration of infused and ethanolic extract of Robusta coffee leaves have anti-diabetic properties and decrease blood glucose levels [16, 17]. Coffee leaves could be a functional food to prevent metabolic syndrome with biochemical contents, including caffeine, chlorogenic acids, flavonoid, and mangiferin [15].

Chlorogenic acids are one of the polyphenol compounds contained in the human diet. It can stimulate glucose absorption in adipose [18]. Chlorogenic acids also can stimulate insulin secretion, increasing glucose tolerance, and inhibit lipid oxidation [18, 19]. Caffeine includes alkaloid compounds in coffee. Caffeine increases the release of insulin from pancreatic β cells and increases glucose tolerance [20]. Flavonoids prevent insulin resistance and increase insulin sensitivity [21], and mangiferin can increase insulin sensitivity and glucose tolerance [22].

This study has determined whether the administration of brewed Robusta coffee leaves processed by Japanese Style Green Tea Process (JGTP) [15] and mangiferin can improve the components of metabolic syndrome when given a 28-days dietary intervention in rats fed with a high-fat-fructose diet (HFFD) for 14 days to mimic human metabolic syndrome. We measured metabolic parameters by assessing insulin levels and the HOMA-IR index.

Material and Methods

This study is included in the research project "Study of The Administration of Brewed Robusta Coffee Leaves for Metabolic Response In Vivo in Metabolic Syndrome", which received funding in 2019 from the Faculty of Medicine, Universitas Diponegoro.

Ethical approval of the study protocol

All experiments were approved by the Ethics Committee of the Medical Research of the Faculty of Medicine, Universitas Diponegoro (No. 20/EC/H/FK-UNDIP/III/2019).

Robusta coffee leaves were processed by JGTP

Robusta coffee leaves were hand-picked from Mekarsari Village, Pasir Jambu, Bandung Regency. Coffee leaves were picked from the second, third, and fourth leaves of each branch of the coffee plants. After that, coffee leaves were processed using the JGTP method. After the leaves were sorted, the blanching process was carried out for 75 seconds.

After the blanching, leaves were dipped in cool water and put in the withering machine for ± 15 minutes while separating the leaves from the midrib to facilitate the crushing process. The crushing process used a crushing-tearing-curling machine (CTC) and was carried out three times to get the best results, like the characteristics of green tea. The last process was the drying process at 80°C for 4-5 hours, for which a rack drier machine (CNC, Sri Lanka) was used so that the water content was between 2-5%. The dried leaves were stored in an airtight sealed container. The coffee leaves were processed in a mini-processing green tea processing laboratory in the laboratory of the Tea and Quinine Research Center Gambung, Bandung-Indonesia.

Rats, diets, and measurements before treatment

Male Wistar rats (6 weeks old, 150-200 g, n = 36) were obtained from the Centre for Food and Nutrition of Universitas Gadjah Mada, Yogyakarta, Indonesia. The rats were individually housed and given standard

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food Comfeed II 20 g/rat/day and water ad libitum. The rats were divided into six groups (n = 6 rats/group): normal control group (K1), metabolic syndrome control group without treatment (K2), mangiferin treatment group (X1), brewed Robusta coffee leaves 0.09 g/200BW group (X2), brewed Robusta coffee leaves 0.18 g/200BW group (X3), brewed Robusta coffee leaves 0.36 g/200BW group (X4).

Besides the K1 group, the other groups were given a high-fat-fructose diet (HFFD), which contains pork oil (20%), cholesterol (1.5%), cholic acid (0.5%) and standard food (80%) given orally, while 1 ml/200gBW of fructose was given through a tube for 14 days. The metabolic syndrome condition was achieved if fasting blood glucose ≥110 mg/dL, triglycerides >150 mg/dL and HDL <40 mg/dL [23, 24].Body weight was measured once a week, and food consumption was measured daily. Fasting blood glucose, triglyceride, and HDL measurements, used as criteria of metabolic syndrome, were carried out after 14 days of HFFD administration. Retro-orbital blood collection was performed before the intervention to measure the insulin level and HOMA-IR index.

Measurements following intervention

Brewed Robusta coffee leaves were made every day and given through a tube. Dried coffee leaves weighed 0.09, 0.18, and 0.36 g/200BW. Each dose of coffee leaves was brewed with 3.6 mL of water at 70˚C for 10 minutes. Mangiferin weighed 20 mg/kg BW and was dissolved with 3.6 mL of water and given through a tube. Measurements of brewed Robusta coffee leaves doses were obtained by conversion from a human dose to a rat dose with 200 g body weight of rat.

The intervention lasted 28 days. On the last intervention day, blood was collected from the retroorbital plexus to measure the insulin level and HOMA IR index. Blood serum was analyzed at the Centre for Food and Nutrition of Universitas Gadjah Mada, Yogyakarta, Indonesia. No rats were dropped out until the end of the intervention.

Statistical analyses

Data were presented as either mean ± SD or median. Paired t-test and one-way analysis of variance (ANOVA) were used for parametric results; differences between the groups were evaluated by the post-hoc test. Wilcoxon, Kruskal-Wallis, and Mann-Whitney U tests were used for non-parametric results. All the statistical analysis was analyzed using the IBM SPSS Statistic v.22 software.

Results

Rats feed intakes

During the treatment period, the intake of experimental rats' food was calculated by weighing food scraps every day individually in all groups.

The percentage of rats' food intake during treatments in metabolic syndrome groups K2, X1, X2, X3, and X4 in the first week was higher than the K1 group. In the second week, the treatment group that had a high percentage of food intake was X2 with 0.09 g/200 g BW dose of brewed Robusta coffee leaves. In the third week, the percentage of food intake in all groups was decreased compared to the previous week. However, in the fourth week, the percentage of food intake in the K2 group was decreased. The X2 group had a higher percentage of food intake compared to the other groups (Figure 1).

Body weight before and after treatments

The average body weight between all groups had increased significantly ($p<0.05$). The treatment groups given HFFD were K2, X1, X2, X3, and X4, as seen in Table 1.

In Table 2, it was shown that there was a significant increase in body weight in all groups ($p<0.05$). The X2 group experienced the highest weight gain after the administration of brewed Robusta coffee leaves with 0.09 g/200 g BW compared to the X3 group and X4 group. In the X1 group with mangiferin 20 mg/kg BW, the rats experienced weight gain. The K1 and K2 groups that were not given any treatment experienced a significant increase in body weight. There were significant differences in body weight between the X1, X2, X3, and X4 groups compared to the K1 and K2 groups, as shown in Table 3. There were no significant differences in body weight between the X4 group compared to the K1 group. The rats gained weight after the administration of mangiferin and brewed Robusta coffee leaves (C. canephora).

Conditioning metabolic syndrome rats

The mean of fasting blood glucose, triglycerides, and HDL levels after the administration of HFFD can be seen in Table 4.

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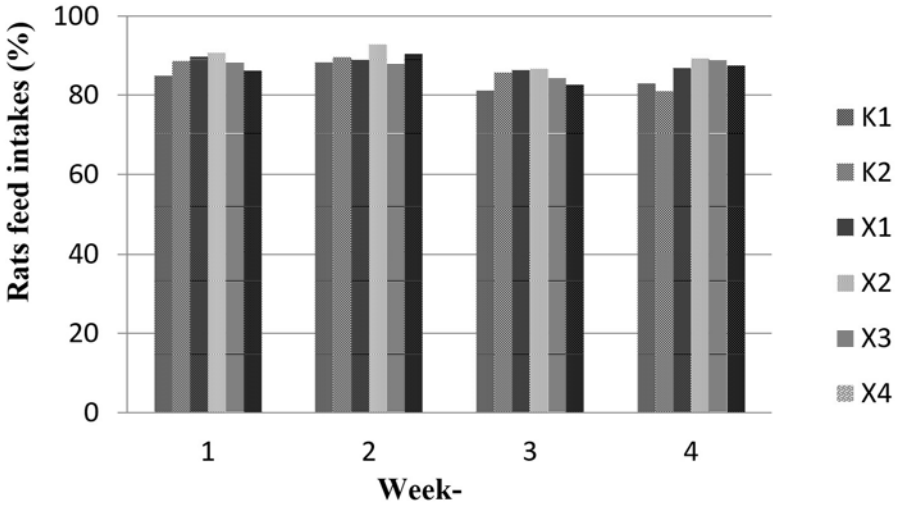

Figure 1: The percentage of rats feed intakes for 4 weeks. Note: normal control group (K1), metabolic syndrome control group without treatment (K2), mangiferin treatment group (X1), brewed Robusta coffee leaves 0.09 g/200BW group (X2), brewed Robusta coffee leaves 0.18 g/200BW group (X3), brewed Robusta coffee leaves 0.36 g/200BW group (X4).

Table 1: The average body weight before and after acquiring metabolic syndrome.

Groups	Body Weight (g)		Δ	p	P
	Pre Mean ± SD	Post Mean ± SD	Mean ± SD		
K1 (n=6)	173.16 ± 3.54	183.00 ± 2.82	9.833 ± 1.16	0.000[a*]	0.001[c*]
K2 (n=6)	179.66 ± 2.25	195.83 ± 2.04	16.16 ± 0.75	0.000[a*]	
X1 (n=6)	180.16 ± 5.98	197.50 ± 5.68	17.33 ± 0.81	0.000a*	
X2 (n=6)	180.16 ± 3.18	196.50 ± 3.78	16.33 ± 1.50	0.000[a*]	
X3 (n=6)	180.83 ± 4.35	198.33 ± 4.67	16.00 (15.00-27.00)[d]	0.026[b*]	
X4 (n=6)	184.66 ± 3.44	201.66 ± 3.32	17.00 ± 1.09	0.000[a*]	

Note: a* = paired t-test, p<0.05 = significantly different; b* = Wilcoxon test, p<0.05 = significantly different; c* = Kruskal-Wallis test, p<0.05 = significantly different; d = abnormal distribution data, displayed in median (min-max).

Table 2: The averageb weight before and after treatment.

Groups	Body Weight (g)		Δ	p	P
	Pre Mean ± SD	Post Mean ± SD	Mean ± SD		
K1 (n=6)	183.00 ± 2.82	208.50 ± 3.27	25.50 ± 1.37	0.000[a*]	0.000[c*]
K2 (n=6)	195.83 ± 2.04	239.33 ± 2.16	43.50 ± 1.64	0.000[a*]	
X1 (n=6)	197.50 ± 5.68	220.50 ± 5.95	22.00 (22.00 – 26.00)[d]	0.024[b*]	
X2 (n=6)	196.50 ± 3.78	230.83 ± 3.37	34.33 ± 1.03	0.000[a*]	
X3 (n=6)	198.33 ± 4.67	226.83 ± 4.99	28.50 ± 1.87	0.000[a*]	
X4 (n=6)	201.67 ± 3.32	228.17 ± 4.70	26.50 ± 1.87	0.000[a*]	

Note: a* = paired t-test, p<0.05 = significantly different; b* = Wilcoxon test, p<0.05 = significantly different; c* = Kruskal-Wallis test, p<0.05 = significantly different; d = abnormal distribution data, displayed in median (min-max)

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Table 3: Mann-Whitney U Test results for weight change before and after treatment.

Groups	Δ BW (g) Mean ± SD	p Value					
		K1	K2	X1	X2	X3	X4
K1	25.50 ± 1.37	-	0.004*	0.026*	0.004*	0.019*	0.219
K2	43.50 ± 1.64		-	0.003*	0.004*	0.004*	0.004*
X1	22.00 (22.00 –26.00)			-	0.003*	0.004*	0.011*
X2	34.33 ± 1.03				-	0.004*	0.004*
X3	28.50 ± 1.87					-	0.122
X4	26.50 ± 1.87						-

Note: *p<0.05 = significantly different.

Table 4: Fasting blood glucose, triglyceride levels and HDL levels after administration of HFFD.

Groups	Fasting blood glucose (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)
	Mean ± SD	Mean ± SD	Mean ± SD
K1 (n=6)	71.29 ± 1.53	68.77 ± 5.97	86.36 ± 2.28
K2 (n=6)	132.70 ± 1.48	157.18 ± 4.88	25.05 ± 1.84
X1 (n=6)	131.81 ± 1.88	153.75 ± 3.11	26.22 ± 1.69
X2 (n=6)	131.56 ± 2.57	156.12 ± 2.48	26.22 ± 1.30
X3 (n=6)	131.14 ± 2.13	153.49 ± 1.96	26.22 ± 2.15
X4 (n=6)	132.53 ± 2.36	156.12 ± 2.77	24.59 ± 1.99

The mean of fasting blood glucose and triglycerides in the K2, X1, X2, X3 and X4 groups have increased compared to the K1 group.

The mean HDL in the K2, X1, X2, X3 and X4 groups have decreased compared to K1. The K2, X1, X2, X3 and X4 groups were given HFFD and experienced metabolic syndrome.

The effect of brewed Robusta coffee leaves and mangiferin administration on insulin levels

Table 5 shows that there were significant mean differences in insulin levels before and after the administration of brewed Robusta coffee leaves and mangiferin in groups K1 (p=0.026), K2 (p= 0.000), X1 (p=0.000), X2 (p=0.001), X3 (p=0.000) and X4 (p=0.000). Significantly increased insulin levels found in treatment groups X2, X3, and X4.

This is proof that these doses of brewed Robusta coffee leaves could increase insulin levels in metabolic syndrome rats. In the X1 group, the insulin levels had increased after the administration of mangiferin. There were significant differences between all groups (p<0.000).

The effects of the administration of brewed Robusta coffee leaves and mangiferin to HOMA-IR Index.

Table 6 shows that there were no significant mean differences in the HOMA-IR index in the K1 group, whereas, in the K2 group, there was a significant HOMA-IR index. The treatment groups with brewed Robusta coffee leaves and mangiferin X1, X2, X3, and X4 had significantly decreased the HOMA-IR index (p<0,000).

Discussion

In this study, the rats acquired metabolic syndrome after the administration of HFFD for 14 days, which

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Table 5: Insulin Levels Before and After Treatment.

Group	Insulin Levels (µIU/ml)		Δ	p	p
	Pre Mean ± SD	Post Mean ± SD	Mean ± SD		
K1 (n=6)	17.35 ± 0.09	17.23 ± 0.09	-0.12 (-0.13 – -0.06)	0.026b*	0.000c*
K2 (n=6)	12.32 ± 0.13	12.20 ± 0.14	-0.12 ± 0.02	0.000a*	
X1 (n=6)	12.30 ± 0.14	15.93 ± 0.12	3.62 ± 0.02	0.000a*	
X2 (n=6)	12.35 ± 0.09	12.94 ± 0.23	0.58 ± 0.21	0.001a*	
X3 (n=6)	12.33 ± 0.10	14.65 ± 0.15	2.32 ± 0.20	0.000a*	
X4 (n=6)	12.45 ± 0.10	15.38 ± 0.08	2.93 ± 0.18	0.000a*	

Note: a* = paired t-test, p<0.05 = significantly different; b* = Wilcoxon test, p<0.05 = significantly different; c* = Kruskal-Wallis test, p<0.05 = significantly different; d = abnormal distribution data, displayed in median (min-max).

Table 6: HOMA-IR Index before and after treatment.

Group	HOMA-IR Index		Δ	p	p
	Pre Mean ± SD	Post Mean ± SD	Mean ± SD		
K1 (n=6)	3.05 ± 0.06	3.07 ± 0.08	0.02 ± 0.03	0.169a*	0.000c*
K2 (n=6)	4.03 ± 0.03	4.07 ± 0.04	0.03 ± 0.03	0.044a*	
X1 (n=6)	4.00 ± 0.09	3.30 ± 0.08	-0.70 ± 0.15	0.000a*	
X2 (n=6)	4.01 ± 0.08	3.50 ± 0.09	-0.51 ± 0.12	0.000a*	
X3 (n=6)	3.99 ± 0.08	3.40 ± 0.11	-0.58 ± 0.17	0.000a*	
X4 (n=6)	4.07 ± 0.05	3.29 ± 0.08	-0.74 (-0.90 – -0.72)	0.027b*	

Note : a* = paired t-test, p<0.05 = significantly different; b* = Wilcoxon test, p<0.05 = significantly different; c* = Kruskal-Wallis test, p<0.05 = significantly different; d = abnormal distribution data, displayed in median (min-max).

increased fasting blood glucose, triglycerides, and decreased HDL levels. The administration of HFFD for 14 days caused rats to have hyperglycemia and dyslipidemia [25]. In the previous study, the administration of HFFD induces weight gain, glucose intolerance, and leptin intolerance. High fat feeding causes hepatic oxidative damage and insulin intolerance [26]. A high-fat diet (HFD) increases fat fatty acids (FFA) concentration and increases the inhibition of insulin signals in peripheral tissue [27]. High fructose feeding causes triglycerides and cholesterol accumulation, which lead to a decrease in insulin sensitivity, insulin resistance, and glucose intolerance [28].

Coffee leaves were processed by the JGTP method. In the previous study using Arabica coffee leaves, the JGTP method produced phenolic components higher than other tea-making processes [15]. The administration of brewed Robusta coffee leaves with the JGTP method increased insulin levels in metabolic syndrome rats.Metabolic syndrome rats had a lower HOMA-IR index after the administration of brewed Robusta coffee leaves. The higher the dose given, the higher the insulin levels and the lower the HOMA-IR index in metabolic syndrome rats. This is related to phenolic components in Robusta coffee leaves, such as caffeine, chlorogenic acids, flavonoid, and mangiferin [15].

In previous studies, the extract of Robusta coffee leaves showed high antioxidant activity, where a higher antioxidant activity was equivalent to the phenolic components in the leaves. The administration of caffeine 0.5g/kg of food for eight weeks in metabolic syndrome rats decreased body fat, increased glucose tolerance, and insulin sensitivity [29]. In another study, caffeine administration (1 g/L) in prediabetes rats progressively increased insulin sensitivity for three weeks. Long-term consumption of caffeine increases

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expression and phosphorylation, some essential proteins that are related to the insulin pathway in the adipose tissue, including GLUT4, insulin receptor, and protein kinase B (Akt) [30].

Caffeine directly inhibits lipid peroxide formation and has a high level of inhibition against radical formation. This compound reduces oxidative stress, ROS, and prevents the antioxidant system [31, 32].

The administration of chlorogenic acids 5 mg/kg BW in metabolic syndrome rats has an anti-diabetic effect [33]. Chlorogenic acids activate the adenosine monophosphate-activated protein kinase (AMPK), a sensor and regulator of cellular energy balance that can inhibit the fatty acid synthesis and production of hepatic glucose.

Therefore, chlorogenic acids with AMPK activation contribute to the regulation of lipid and glucose metabolism [34]. Chlorogenic acid has a hypoglycemic effect and insulin-sensitizing, which is able to increase glucose metabolism in diabetic patients [35]. The administration of chlorogenic acids in diabetic rats at a dose of 5 mg/kg BW for 45 days caused a decrease in lipid oxidation and increased endogenous antioxidant enzymes [33].

Flavonoids act as antioxidants and insulin secretagogue. Flavonoids trigger and strengthen the insulin secretion pathway in β cells [36]. The provision of 1 mg/kg of genistein in high-fructose fed rats for two weeks improved insulin resistance by restoring homeostatic model assessment for insulin resistance (HOMA-IR) [37]. The administration of green tea extract (1-2 g/kg) in high-fat-fructose fed rats for six weeks increased mRNA levels in Insulin Receptor Substrate 1 (IRS1) and GLUT4, which is caused by improved insulin resistance in skeletal muscle tissue [38]. High anthocyanin consumption is associated with significantly lower HOMA-IR [39].

Another compound suspected in Robusta coffee leaves is mangiferin. Mangiferin has great potential in treating metabolic syndrome. Mangiferin decreases weight gain in fat mass. This compound can increase the uses of oxygen and energy expenditure with stimulation of carbohydrate use in skeletal muscles. Mangiferin reduces glucose plasma, increases insulin sensitivity, and glucose tolerance [22].

The administration of 5% mangiferin in high-fat-fed rats modulates glucose and insulin, resulting in improved glucose and insulin tolerance. This compound is able to reduce oxidative stress by decreasing malondialdehyde (MDA) levels and increasing glutathione (GSH) and superoxide dismutase enzyme (SOD) [40]. The provision of mangiferin 40 mg/kg BW in diabetic rats increased insulin resistance by decreasing pancreatic β cell damage, regenerating insulin resistance, and decreasing blood glucose [41–43].

Conclusions

Brewed Robusta coffee leaves processed by the JGTP method increase insulin levels and decrease the HOMA-IR index in high-fat-fructose-induced metabolic syndrome in rats. The administration of brewed Robusta coffee leaves has the same effect as the administration of mangiferin in metabolic syndrome rats.

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Conflict of Interest

The author declares no conflict of interest.

References

1. Srikanthan K, Feyh A, Visweshwar H, Shapiro JI, Sodh K. Systematic Review of Metabolic Syndrome Biomarkers: A Panel for Early Detection, Management, and Risk Stratification in the West Virginian Population. Int J Med Sci 13(1): 25–38, 2016.
2. Zujko ME, Waśkiewicz A, Witkowska AM et al. Dietary Total Antioxidant Capacity and Dietary Polyphenol Intake and Prevalence of Metabolic Syndrome in Polish Adults: A Nationwide Study. Oxid Med Cell Longev 2018: 1–10, 2018.
3. Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 285(19): 2486–97, 2001.
4. Hurrle S, Hsu WH. The etiology of oxidative stress in insulin resistance. Biomed J. 40(5): 257–62, 2017.
5. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444(7121): 840–6, 2006.
6. Marseglia L, Manti S, D'Angelo G et al. Oxidative Stress in Obesity: A Critical Component in Human Diseases. Int J Mol Sci 16: 378–400, 2015.
7. Balsan GA, Vieira JL da C, Oliveira AM de, Portal VL. Relationship between adiponectin, obesity and insulin resistance. Rev Assoc Med Bras (1992) 61(1): 72–80, 2015.

https://doi.org/10.46389/rjd-2020-1004
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8. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes 6(3): 456–80, 2015.
9. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4(8): 118–26, 2010.
10. Yadav A, Kumari R, Yadav A, Mishra JP, Srivatva S, Prabha S. Antioxidants and its functions in human body - A Review. Res Environ Life Sci 9(11): 1328–31, 2016.
11. Mironczuk-Chodakowska I, Witkowska AM, Zujko ME. Endogenous non-enzymatic antioxidants in the human body. Adv Med Sci 63(1): 68–78, 2018.
12. Herlyana E. Fenomena Coffee Shop Sebagai Gejala Gaya Hidup Baru Kaum Muda. ThaqÃfiyyÃT 13(1): 188–204, 2012.
13. Rasyid R, Sanjaya WF, Zulharmita. Penetapan Kadar Kofein Daun Kopi Kawa (Coffea Robusta ,Lind). J Farm Higea 5(2): 137-143, 2013.
14. Muttalib, Abdul S, Karyadi, Wahyu JN, Bintoro, Nursigit. Identifikasi Aroma Campuran (Blending) Kopi Arabika Dan Robusta Dengan Electronic Nose Menggunakan Sistem Pengenalan Pola. Semin Nas Perhimpun Ahli Tek Pertan 154–63, 2012.
15. Chen X-M, Ma Z, Kitts DD. Effects of processing method and age of leaves on phytochemical profiles and bioactivity of coffee leaves. Food Chem 249: 143–53, 2018.
16. Shiyan S, Herlina, Arsela D, Latifah E. Aktivitas Antidiabetes Ekstrak Etanolik Daun Kopi Robusta (Coffea canephora) Pada Tikus Diabetes Tipe 2 Yang Diberi Diet Lemak Tinggi Dan Sukrosa. J Farm Sains dan Prakt III(2), 2017.
17. Purwaningsih S. Pengaruh Pemberian Daun Kopi (Coffea robusta Lindl)Terhadap Kadar Gula Darah Mencit Akibat Diinduksi Aloksan. Vet Med 7(3), 2014.
18. Meng S, Cao J, Feng Q, Peng J, Hu Y. Roles of Chlorogenic Acid on Regulating Glucose and Lipids Metabolism: A Review. Evid Based Complement Altern Med 2013: 1–11, 2013.
19. Santana-Gálvez J, Cisneros-Zevallos L, Jacobo-Velázquez DA. Chlorogenic Acid: Recent Advances on Its Dual Role as a Food Additive and a Nutraceutical against Metabolic Syndrome. Molecules 22: 358, 2017.
20. Başpınar B, Eskici G, Özçelik AÖ. How coffee affects metabolic syndrome and its components. Food Funct 8: 2089–101, 2017.
21. Galleano M, Calabro V, Prince PD et al. Flavonoids and metabolic syndrome. Ann N Y Acad Sci 1259(1): 87–94, 2012.
22. Ekaterina, Fomenko V, Chi Y. Mangiferin Modulation of Metabolism and Metabolic Syndrome. Biofactors 42(5): 492–503, 2016.
23. Rochlani Y, Pothineni NV, Kovelamudi S, Mehta JL. Metabolic syndrome: pathophysiology, management, and modulation by natural compounds. Ther Adv Cardiovasc Dis 11(8): 215–25, 2017.
24. Rohman MS, Lukitasari M, Nugroho DA, Nashi W, Nugraheini NIP, Sardjono TW. Development of an Experimental Model of Metabolic Syndrome in Sprague Dawley Rat. Res J Life Sci 4(1): 76–86, 2017.
25. Octavia ZF, Djamiatun K, Suci N. Pengaruh pemberian yogurt sinbiotik tepung pisang tanduk terhadap profi l lipid tikus sindrom metabolic. J Gizi Klin Indones 13(4): 159–69, 2017.
26. Jarukamjorn K, Jearapong N, Pimson C, Chatuphonprasert W. A High-Fat, High-Fructose Diet Induces Antioxidant Imbalance and Increases the Risk and Progression of Nonalcoholic Fatty Liver Disease in Mice. Scientifica (Cairo). 2016: 10, 2016.
27. Matsuda Y, Kobayashi M, Yamauchi R et al. Coffee and caffeine improve insulin sensitivity and glucose tolerance in C57BL/6J mice fed a high-fat diet. Biosci Biotechnol Biochem 75(12): 2309–15, 2011.
28. Wong SK, Chin K-Y, Suhaimi FH, Soelaiman I-N, Fairus A. Animal Models of Metabolic Syndrome: A Review. Nutr Metab (Lond) 13(65): 1–12, 2016.
29. K.Panchal S, Wong W-Y, Kauter K, Ward LC, Brown L. Caffeine attenuates metabolic syndrome in diet-induced obese rats. Nutrition. 28(10): 1055–62, 2012.
30. Coelho JC, Melo BF, Rodrigues T et al. Caffeine Restores Insulin Sensitivity and Glucose tolerance in High-sucrose Diet Rats: Effects on Adipose Tissue. J Cardiovasc Dis 440–9, 2016.
31. Tellone E, Galtieri A, Giardina B et al. A Focus on Human Red Blood Cells and Correlations with Several Neurodegenerative Disorders. In: Coffee in Health and Disease Prevention. Victor R Preedy (ed). pp: 835–42, 2015.
32. Jeszka-Skowron M, Sentkowska A, Pyrzyńska K, Peña MP De. Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation. Eur Food Res Technol 242: 1403, 2016.
33. Pari L, Karthikesan K, Menon VP. Comparative and combined effect of chlorogenic acid and tetrahydrocurcumin on antioxidant disparities in chemical induced experimental diabetes. Mol Cell Biochem 341(1–2): 109–17, 2010.
34. Buscemi S, Marventano S, Antoci M et al. Coffee and Metabolic Impairment: An Updated Review of Epidemiological Studies. NFS J 3: 1–7, 2016.
35. Bhandarkar NS, Brown L, Panchal SK. Chlorogenic acid attenuates high-carbohydrate, high-fat diet–induced cardiovascular, liver, and metabolic changes in rats. Nutr Res 62: 78–88, 2019.
36. Russo B, Picconi F, Malandrucco I, Frontoni S. Flavonoids and Insulin-Resistance: From Molecular Evidences to Clinical Trials. Int J Mol Sci 20(2061):1–18, 2019.
37. Palanisamy N, Viswanathan P, Anuradha. CV. Effect of Genistein, a Soy Isoflavone, on Whole Body Insulin Sensitivity and Renal Damage Induced by a High-Fructose Diet. Ren Fail 30(6): 645–54, 2008.
38. Cao H, Hininger-Favier I, Kelly MA et al. Green Tea Polyphenol Extract Regulates the Expression of Genes Involved in Glucose Uptake and Insulin Signaling in Rats Fed a High Fructose Diet. J Agric Food Chem 55(15): 6372–8, 2007.
39. Jennings A, Welch AA, Spector T, Macgregor A, Cassidy A. Intakes of Anthocyanins and Flavones Are Associated with Biomarkers of Insulin Resistance and Inflammation in Women. J Nutr 144(2): 202–8, 2014.
40. Sun H, Wu C, Tang J, Li M, Zhou C. The Effects of Mangiferin On Metabolic Syndrom: The Collaborative Meta-Analysis Combing With Animal Experiments. World J Pharm Pharm Sci 7(12): 236–67, 2018.
41. Sellamuthu PS, Arulselvan P, Muniappan BP, Kandasamy M. Effect of mangiferin isolated from Salacia chinensis regulates the kidney carbohydrate metabolism in streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed 2(3): S1583–7, 2012.

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42. Sellamuthu PS, Muniappan BP, Arulselvan P, Fakurazi S. Mangiferin from Salacia chinensis prevents oxidative stress and protects pancreatic β-cells in streptozotocin-induced diabetic rats. J Med Food 16(8): 719–27, 2013.

43. Sellamuthu PS, Fakurazi S, Arulselvan P, Kandasamy M. Beneficial effects of mangiferin isolated from Salacia chinensis on biochemical and hematological parameters in rats with streptozotocin-induced diabetes. Pak J Pharm Sci 27: 161–7, 2014.

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Original Article

The Influence of Diabetic Family History on the Development of Neuropathy in Type 2 Diabetes Patients: A Survival Analysis

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Abstract

Introduction: Neuropathy is a common and dangerous complication of diabetes mellitus with the highest mortality rate, as well as high costs for diabetic patients. This study aimed to identify the factors affecting the event time of neuropathy incidence in diabetic patients using cure models. **Material and Methods:** For data collection, all the patients whose diabetic screening tests conducted by the Fereidounshahr Health Centers and were negative in 2006, but were diagnosed with diabetes mellitus in 2007 through rescreening and were at least 30 years of age were enrolled in the study, and their neuropathy status was monitored for at least ten years. Mixture and non-mixture cure models were used for data analysis, and the survival-specific receiver operating characteristic curve was also used to compare the efficiency of the models. **Results:** Of the 371 diabetic patients, 257 were females (69.3%), and 178 (47.97%) had a family history of diabetes mellitus. Having fitted the exponential mixture and non-mixture cure models, as well as Weibull, log-normal, and log-logistic ones, we found the log-logistic model as being the most efficient. In the final fitted log-logistic mixture cure model, the variables such as a family history of diabetes mellitus, fasting blood sugar, ethnicity, and gender were significantly associated with the time of neuropathy incidence. **Conclusion:** The results of this study showed that female patients from Fars who had a positive family history of diabetes mellitus needed to more precisely control their fasting blood sugar in order to delay the incidence of neuropathy.

Keywords: Cure Models, Survival Analysis, Type 2 Diabetes Mellitus, Diabetic Neuropathy.

Introduction

Diabetes mellitus (DM) is one of the most common non-communicable diseases in the world and the Eastern Mediterranean Region (in which Iran is also located). DM is known as the leading cause of blindness, kidney failure, lower limb amputation, and death in the electronic medical record (EMR). Diabetic foot is one of the most common complications of DM that is often overlooked. Not only the costs of caring and treating diabetic foot problems are high due to the increased likelihood of hospitalization and amputation, but also, the disease has a high burden [1]. An estimation of the burden of DM and its complications based on the lost years in Iran, indicated in 2001 that neuropathy, diabetic foot, and amputation accounted for 18% of the total DM burden altogether [2]. The prevalence of diabetic foot is 4.6-12% in the world [3, 4] and 3% in the Iranian diabetic population. Neuropathy is one of the most common microvascular complications of DM observed in both type 1 and type 2 DM. The incidence of polyneuropathy in diabetic patients is 11-31%. Severe pain that decreases and can lead to loss of sensation, increased risk of foot ulcers and amputation are

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among the complications of diabetic neuropathy [5] with many negative effects on the patients' quality of life. Previous studies reported that neuropathic pain was present in 33-47% of diabetic patients. According to the information obtained, the pain gets severe at night and rest and may lead to insomnia, restlessness, and many other problems for the patients. The incidence of neuropathy and its severity depend on the duration of DM [6]. Semi-parametric and parametric models can be used to determine the factors influencing the event time of neuropathy incidence in patients with type 2 DM. The condition for using such models is the gradual failure of all the subjects under investigation for a sufficiently long time. For example, in chronic diseases that are usually incurable and continue to progress, all the patients are expected to experience the incidence gradually, but this condition is not always met, and a certain proportion of patients may not experience the intended incidence during the follow-up period, in which case, common parametric and semi-parametric models cannot be used. For instance, not every patient rejects a transplanted organ or not everyone infected with the Human Immunodeficiency Virus (HIV) gets AIDS. Generally, in survival studies, cure models are used if a percentage of the population is immune to the incidence. In this case, the people are divided into two groups of susceptible and non-susceptible. The people in the susceptible group have long-term survival and are immune to the incidence in question [7, 8]. Hence, a cure model can be used, the most important objectives of which are to estimate the proportion of cured (immunized) individuals, to estimate the survival function for susceptible and at-risk groups, and to examine the factors affecting the aforementioned items [8, 9]. It should be noted that the high censorship rate in the survival data causes biased estimates of maximum likelihood in standard survival models of various types [10]. However, these models are not capable of separating the factors affecting short- and long- term survival. The use of cure models is recommended to analyze the survival of patients separately. As long- and short-term survival is examined in these models, censorship rates may be too low or too high. With a censorship rate of > 0.6 for patients with at least one diabetic first-degree relative and a censorship rate of 0.4 for other patients (the survival rates of whom are shown separately in Figure 1), the need for using mixture and non-mixture cure models seems obvious. Thus, the aim of this study was to investigate the effect of family history of DM on the event time of neuropathy in type 2 DM patients using mixture and non-mixture cure models.

Material and Methods

In order to collect the data, all the people whose DM screening tests were conducted at the Fereidounshahr health centers and were negative in 2006, but were di-

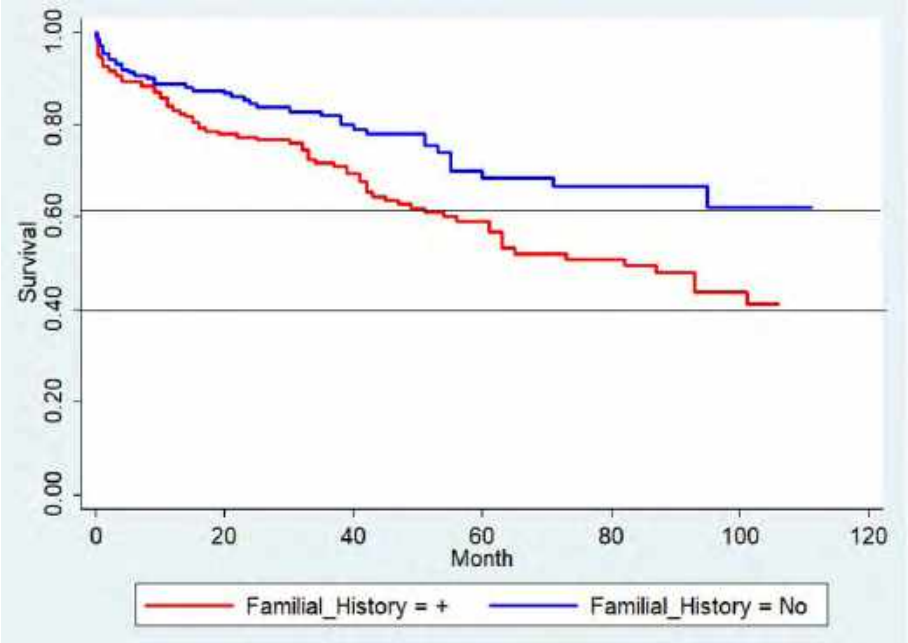

Figure 1: Estimation of survival time in the absence of neuropathy in patients studied by the history of diabetes mellitus in first-degree family members.

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agnosed with type 2 DM in 2007 through rescreening, and were at least 30 years of age, were included in the study, and their neuropathy status was monitored for at least ten years.

In this survival study, the response variable was the time from the diagnosis of DM until the diagnosis of neuropathy. The failure time was by month, and censored cases included those who had not been diagnosed with neuropathy at the end of the study, those who died, and those missing at the follow-up (migrants) before the end of the study. The data on demographic variables including gender, age, occupation, education, place of residence, race, history of DM in first-degree relatives, DM treatment method, smoking, along with laboratory variables including fasting blood sugar, height, weight, body mass index (BMI), total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, creatinine, HbA1c, diastolic and systolic blood pressure, as well as the clinical diagnostic and questionnaire variables of neuropathy and neuropathy diagnosis using nerve conduction studies were recorded through reviewing the patients' records and interviewing them if necessary.

The Akaike information criterion was used to fit the models. The criterion is defined as Akaike Information Criteria (AIC)= -2L (b)+2p, where p is the number of parameters in the model, and the smaller the criterion is, the more appropriate the proposed model will be. L is also defined as the likelihood function for b parameters.

The survival-specific receiver operating characteristics (ROC) curve is one of the methods used to evaluate fitted survival models. It is a benchmark for measuring model efficiency by measuring the area under the ROC curve. The curve graphically shows the correct sensitivity or prediction against false prediction [11]. The Area Under Curve (AUC) was used to compare the accuracy of the models. The closer its value was to 1 or the greater the area under the curve, the higher the accuracy of the model would be. In this study, exponential mixture and non-mixture cure models, as well as Weibull, log-logistic and log-normal, were used.

Cured models

Cured models are divided into two general categories:

- Mixture models: As the name implies, a mixture model is a combination of total survival for both cured and uncured patients. The former group consists of patients who are immune to the incidence. They are considered cured. The latter consists of the patients who are not immunized to the incidence at all and are at risk until the end of the study. The survival function for mixture cure models is defined as [12]:

$$S_p(t) = P + (1 - P)S^*(t), S^*(\infty) = 0, S_p(\infty) = P > 0$$

In this model, the p ratio of the cured or immunized individuals can be modeled by logistic regression, probit function, complementarity log-log link function, and linear regression. It should be noted that cure models are the standard survival models in the absence of immunized individuals.

- Non-mixture Models: These models are defined for populations with an incomplete distribution function of the survival time for the whole population, i.e., the value of the cumulative distribution function does not reach one. The survival function for these models is defined as:

$$S(t) = p^{(1-S(t))}$$

In these populations, the difference between the cumulative distribution function and the value 1 shows the proportion of cured people; so, in these models, the proportion of cured individuals is within the model and can be directly obtained by having an infinite survival limit. If the data under study are i=1,...,n (t_i, δ_i, X_i, Z_i) (where the values of 1 and 0 for δ_i indicating the incidence of the event and the censorship, respectively, t_i indicates the survival time, and X_i and Z_i represent the variables related to the survival rate and cure probability, respectively), the likelihood function for the i^{th} person will be as follows:

$$if \quad \delta_i = 1 \Rightarrow L = \pi_i(Z_i) f(t_i|U = 1, X_i)$$
$$if \quad \delta_i = 0 \Rightarrow L = 1 - \pi_i(Z_i) + \pi_i(Z_i) S(t_i|U = 1, X_i)$$

In the formula, $f = S\lambda$ and is the probability density function T. Thus, the likelihood function will be as follows:

$$L(\gamma, \beta) = \prod^{n} [(1 - \pi_i(Z_i) S(t_i|U = 1, X_i))]^{1-\delta_i} \times [\pi_i(Z_i) f(t_i|U = 1, X_i)]^{\delta_i}$$

Results

Of the 371 DM patients, 257 were females (69.3%), and 178 (47.97%) had a family history of DM. Table 1

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Table 1: Comparison of descriptive of demographic and laboratory variables.

Variables	Neuropathy + Frequency (%)	Neuropathy NO Frequency (%)	Statistic X^2	P-Value
Gender				
Male	26 (22.4%)	88 (34.5%)	5.480	0.019
Female	90 (77.6%)	167 (65.5%)		
Race				
Georgia	36 (31%)	101 (39.8%)	9.263	026
Bakhtiari	32 (27.6%)	85 (33.5%)		
Persia	18 (15.5%)	19 (7.5%)		
Turkey	30 (25.9%)	49 (19.3%)		
Job activity				
Low	15 (12.9%)	42 (16.5%)	2.010	0.366
Moderate	85 (73.3%)	168 (65.9%)		
High	16 (13.8%)	45 (17.6%)		
Family History				
Yes	74 (64.3%)	104 (44.4%)	12.223	<0.001
No	41 (35.7%)	130 (55.6%)		
Treatment				
Oral	85 (73.3%)	222 (87.1%)	20.813	<0.001
Insulin	13 (11.2%)	15 (5.9%)		
Both	18 (15.5%)	11 (4.3%)		
No medication	0 (0.0%)	7 (2.7%)		
	Mean (± s.d)	**Mean (± s.d)**	**statistic t**	**P-Value**
FBS	172.25 (± 55.170)	164.57 (± 54.762)	-1.971	0.048
BMI	28.36 (± 4.485)	28.52 (± 4.201)	0.310	0.757
Hb1Ac	8.15 (± 1.631)	7.71 (± 1.919)	-2.040	0.420
Cholesterol	200.98 (± 46.347)	192.38 (± 46.953)	-1.586	0.114
Triglyceride	196.74 (± 124.232)	193.15 (± 108.830)	-0.272	0.786
HDL	49.37 (± 22.022)	48.44 (± 16.892)	-0.404	0.687
LDL	112.6 (± 42.897)	110.16(± 40.185)	-0.500	0.617
BUN	16.42 (± 7.836)	15.89 (± 6.462)	-0.640	0.523
Creatinine	0.79(± 0.241)	0.82 (± 0.291)	0.987	0.324

Note: s.d: standard deviation. Abbreviations: BMI, Body Mass Index; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; BUN, Blood Urea Nitrogen; FBS, Fasting Blood Sugar

shows demographic data and laboratory results of the included patients by the final neuropathy status.

According to the results of the chi-square test, the number of diabetic women with neuropathy was three times as much as that of the men. Also, there was a significant difference between Georgian, Bakhtiari, Persian, and Turkish ethnic groups in terms of the number of neuropathy patients. In other words, of the 37

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Persian subjects, 18 (49%) had neuropathy, and the proportion was 26%, 27%, and 38% in Georgian, Bakhtiari, and Turkish ones, respectively. In this study, no significant relationship was found between occupational activity and suffering from neuropathy, but family history and treatment type had a significant relationship with neuropathy. The independent t-test also showed a significant relationship between fasting blood sugar and neuropathy (P-value = 0.048), but no significant relationship was found between BMI, HbA1c, creatinine, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and blood urea nitrogen (BUN) (P-value <0.05).

The one-year, three-year, and five-year survival rates are presented in Table 2. The five-year survival rate was 0.64 in the studied patients, necessitating the use of a cure model (Table 2).

In the survival analysis with an auxiliary variable (single regression) for the four models used as mixture and non-mixture, the family history of diabetes, fasting blood sugar, ethnicity, and gender were significant.

The results of the mixture and non-mixture exponential, Weibull, log-logistic and log-normal cure models obtained through single and multiple regression, respectively, are shown in Table 3.

In the final model, the Akaike values were 1469.8 (1467.4), 1360.8 (1371.4), 1347.3 (1357.6), and 1349.2 (1361.8), respectively, amongst which the log-logistic mixture model with the lowest Akaike value had the best fitness. The results of the multiple regression for log-logistic and Weibull mixture cure models are shown in Table 4. In fitting the two models, the cure fraction with Fasting Blood Sugar (FBS) and family history of diabetes, and the scale section with ethnicity and gen-

Table 2: One-year, three-year and five-year survival rates and 95% confidence intervals in the studied patients.

Survival	Survival rate	S.d	95% Confidence Interval	
			Lower bound	Upper bound
One-year	0.86	0.018	0.828	0.898
3-year	0.77	0.023	0.723	0.817
5-year	0.64	0.031	0.582	0.704

Table 3: Results of the mixture and non-mixture exponential, Weibull, log-logistic, and log normal single regression cure model in the studied patients.

Model	exponential		Weibull		log-normal		log-logistic	
Variable	mixture	non-mixture	mixture	non-mixture	mixture	non-mixture	mixture	non-mixture
-	1281.86	1281.51	1264.35	1269.56	1282.46	-	1272.23	1270.83
Gender	1278.08	1278.66	1260.40	1265.32	1280.44	-	1268.88	1264.88
Age	1274.05	1272.48	1256.33	1255.03	1274.46	-	1264.05	-
FBS	1283.85	1281.81	1266.34	1265.10	1283.28	-	1273.62	1266.62
Job activity	1283.66	1283.42	1266.24	1271.54	1284.40	-	1274.07	1272.80
Tobacco	1281.91	1281.00	1263.90	-	1280.55	-	1270.86	-
Education	1283.24	1283.02	1265.86	1271.06	1283.90	-	1273.76	-
Zone	1283.30	1282.08	1265.70	1268.33	1283.72	-	1273.60	-
Race	1281.86	1281.51	1258.96	1263.86	1281.72	1293.00	1267.36	1267.60
Family history	1281.86	1281.51	1271.12	1275.92	1315.56	1329.82	1277.20	1281.01
Treatment	1283.82	1283.28	1265.54	1270.82	1283.21	-	1273.20	-

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Heart Disease	1280.13	1277.20	1262.04	-	1280.00	-	1259.66	-
BMI	1253.92	1254.74	1235.72	-	1252.36	-	1242.60	-
Cholesterol	1253.12	1252.04	1241.72	1242.26	1239.16	-	1248.50	-
Triglyceride	1264.45	1263.10	1250.29	1253.60	1265.24	-	1257.67	1254.51
HDL	1020.08	1017.16	998.20	-	1010.44	-	1003.66	-
LDL	1238.09	1237.47	1225.03	1228.73	1238.88	-	1231.92	-
BUN	1218.01	1214.19	1199.10	-	1214.96	-	1205.60	-
Creatinine	1213.12	1212.95	1198.91	-	1217.12	-	1206.75	-
HbA1c	1238.02	1236.19	1222.82	-	1238.40	-	1229.29	1222.66
BP sys	1115.58	1114.14	1094.20	-	1108.86	-	1100.75	-
BP dia	1114.48	1112.76	1094.05	1090.58	1109.10	-	1101.02	-

Abbreviations: BMI, Body Mass Index; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; BUN, Blood Urea Nitrogen; FBS, Fasting Blood Sugar; BP sys, Blood Pressure systolic; BP dia, Blood Pressure diastolic; VEGGF, Vascular Endothelial Growth Gene Factor.

Table 4: Results of multiple analysis of logistic and Weibull mixture of the cure models.

	coefficient		**s.d**		**z**		**p-value**	
Variables	**Log-Logistic**	**Weibull**	**Log-Logistic**	**Weibull**	**Log-Logistic**	**Weibull**	**Log-Logistic**	**Weibull**
Cure fraction								
FBS	0.005	0.005	0.0025	0.0023	1.970	2.090	0.049	0.037
Family history								
No (base)								
Yes	1.179	1.033	0.3218	0.2959	3.660	3.490	<0.001	<0.001
Scale								
Gender								
Male (base)								
Female	3.033	3.006	0.3569	0.2878	8.500	10.450	<0.001	<0.001
Race								
Georgia (base)								
Bakhtiari	-1.239	-2.105	0.5227	0.4408	-2.370	-4.780	0.018	<0.001
Persian	0.678	-0.123	0.6990	0.5661	0.970	-0.220	0.332	0.827
Turk	-1.012	-2.073	0.5505	0.4159	-1.840	-4.980	0.066	<0.001
Shape								
Gamma	-0.300	-0.627	0.0933	0.0763	-3.220	-8.220	0.001	<0.001

Abbreviations: FBS, Fasting Blood Sugar.

der were significant. Given that the variables of fasting blood sugar and family history of diabetes were significant in the cure fraction, these two variables divided the subjects into cured and uncured groups. According to the results, females developed neuropathy faster than males, and Persians faster than other ethnic groups.

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It was found out in the fitted log-logistic model that for 50 units of increase in fasting blood sugar, the patients were 1.3 times more likely to have neuropathy (uncured). Also, those with a positive family history of DM were over three times more likely to have neuropathy, but in the non-susceptible group, females were at risk of neuropathy for about 21 times as much as males. Besides, Persians got neuropathy faster than other ethnicities.

Figure 2 shows the AUC values to compare the efficiency of the Weibull and log-logistic mixture cure models. The AUC values of the log-logistic mixture cure model were always greater than those of the Weibull model so that the Wilcoxon test showed a significant difference between these values (P-value <0.001), indicating greater efficiency of the log-logistic model in survival estimation.

Discussion

DM reduces the body's speed and ability to utilize and metabolize glucose completely. As a result, blood sugar increases, resulting in hyperglycemia. If the increase in blood sugar lasts for a long time (i.e., increased time of DM occurrence) [14, 15], macrovascular complications (atherosclerosis) will begin to damage the arterial walls. Damage to the walls of the arteries may lead to chronic vascular inflammation, immune cell infiltration, lipid deposition of LDL particles into the vessels, and smooth muscle extension [16]. Microvascular complications of DM are caused by the destruction of very small veins in the body that can affect various parts of the body, such as kidneys, eyes, and nerves.

Long-term hyperglycemia causes the destruction of peripheral nerve cells. These cells are at higher risk because they are unable to regulate blood glucose uptake in the long run and lead to neuropathy over some years [17–19]. Increased blood glucose causes glucose accumulation inside the nerve cells and converts to sorbitol and fructose over time [20], which, in turn, impairs axonal transport, nerve membrane fragility, and ultimately leads to nerve cell destruction [21]. In this study, FBS and HbA1c variables were significant in single regression, but FBS was finally significant as a determinant of susceptible and immunized groups in the multiple regression.

A family history of DM in first-degree relatives was one of the most critical risk factors for the incidence of neuropathy in this study (P-value <0.001). The ratio of neuropathy was higher in those with a family history of DM than other patients [22-25]. Based on the aforementioned results, it can be hypothesized that genetic factors can affect the incidence of neuropathy [24, 25]. Numerous studies also emphasized the role of genetics

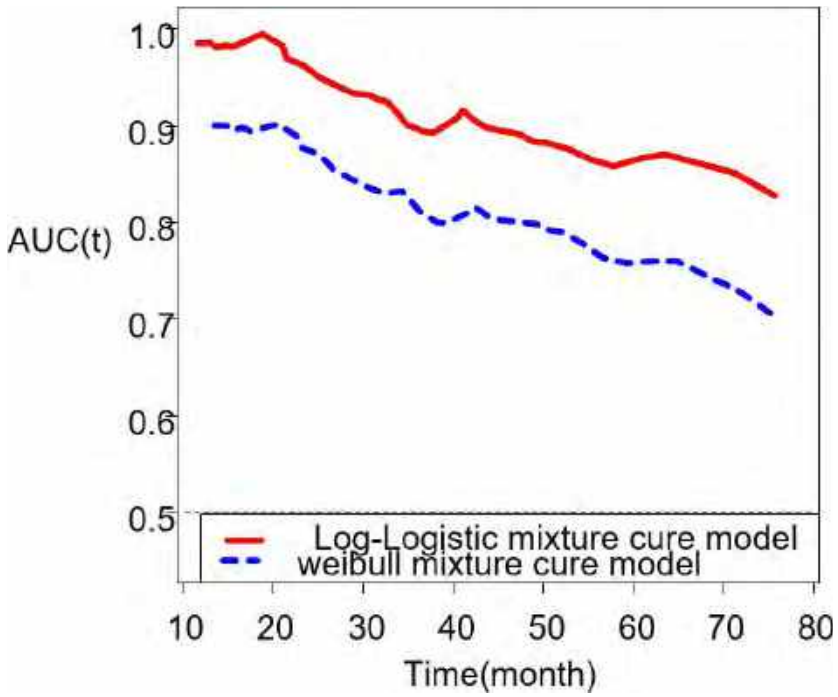

Figure 2: Comparison of accuracy between the Area Under Curve (AUC) of Receiver Operating Characteristics Curve (ROC) of Log-Logistic mixture cure model and Weibull mixture cure model.

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- Vascular Endothelial Growth Gene Factor (VEGGF) - in the development of neuropathy [26–24].

The difference between males and females in terms of developing neuropathy could be attributed to hormonal imbalances in the two genders. Endocrine disorders result in hormonal imbalance and neuropathy. Inadequate production of thyroid hormones, for example, slows down the metabolism and leads to fluid retention and tissue swelling, which can put pressure on peripheral nerves. In contrast, overproduction of thyroid hormones can cause acromegaly and abnormal enlargement of bones and joints, trapping the adjacent nerves and causing neuropathic pain [27]. Some studies have proved the role of progesterone in the regeneration of damaged nerve myelin membrane [28]. In the present study, gender was identified as an effective predictor of developing neuropathy, i.e., females developed neuropathy faster than males.

Conclusion

According to the results of this study, it can be concluded that female patients, Persians, and those with a positive family history of DM needed to have more precise control of fasting blood sugar in order to delay neuropathy and increase the time of developing it.

Considering the efficiency of the mixture log-logistic cure model, it can be concluded that as the model is initially incremental and then decreasing, many uncured patients with DM experience neuropathy at the onset of the disease, and then a decrease in the number of neuropathy cases can be seen [29].

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Khatib O, Malazy OT. Prevention and public approach to diabetic foot. Journal of Diabetes and Metabolic Disorders 7: 1, 2007. [in persian]
2. Abolhasani F, Tabataei O, Tehrani MRM, Larijani B. Burden of diabetes and its complications in Iran in year 2000. Iranian Journal of Diabetes and Metabolism 5(1): 35-48, 2005. [in persian]
3. Songer TJ, Zimmet PZ. Epidemiology of type II diabetes. Pharmacoeconomics 8(1): 1-11, 1995.
4. Wild S et al. Global burden of diabetes mellitus in the year 2000.
5. Pajouhi M, Shaban NK, Mohajeri T. Evaluation and prevention of diabetic neuropathy. Tehran University Medical Journal TUMS Publications 65(3): 1-6, 2007. [in persian]
6. Pourmomeny AA, Safaei H, Amini M, Hassanzadeh A. The effect of on pain relief in patient with diabetic neuropathy type II. Iranian Journal of Endocrinology and Metabolism 11(4), 2009 [in persian]
7. Rahimzadeh K, Hajizadeh E, Feyzi S. Assessment of factor effectiveness on the bilateral corneal graft rejection in the keratoconus with cure frailty model. Research in Medicine 34(2): 117-122, 2010. [in persian]
8. Maller RA, Zhou X. Survival analysis with long-term survivors. John Wiley & Sons, 1996.
9. Farewell VT. The use of mixture models for the analysis of survival data with long-term survivors. Biometrics 38(4): 1041-1046, 1982.
10. Yu H-F, Peng C-Y. Estimation for Weibull distribution with type II highly censored data. Quality Technology & Quantitative Management 10(2): 193-202, 2013.
11. Heagerty PJ, Lumley T, Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics 56(2): 337-344, 2000.
12. Corbière F, Joly P. A SAS macro for parametric and semiparametric mixture cure models. Computer methods and programs in biomedicine 85(2): 173-180, 2007.
13. Othus M, Barlogie B, Leblanc ML, Crowley JJ. Cure models as a useful statistical tool for analyzing survival. Clinical Cancer Research 18(14): 3731-3736, 2012.
14. Pirart J. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973 (3rd and last part) (author's transl). Diabete & metabolisme 3: 245-56, 1997.
15. Adler AI, Boyko EJ, Ahroni JH, Stensel V, Forsberg RC, Smith DG. Risk factors for diabetic peripheral sensory neuropathy: results of the Seattle Prospective Diabetic Foot Study. Diabetes care 20(7): 1162-1167, 1997.
16. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clinical diabetes 26(2): 77-82, 2008.
17. Stolar M. Glycemic control and complications intype 2 diabetes mellitus. Am J Med 123(3Suppl): S3-11, 2010.
18. Chao CC ,Hsieh SC, Yang WS et al. Glycemic control is related to the severity of impaired thermal sensations in type2 diabets. Diabetes Metab Res Rev 23(8):612-20, 2007.
19. Abrahams M. Consequences of Elevated HbA1c in type2 Diabetes. Available in http://www.medpagetoday.com/resource_center/g1p1_type_2_diabetes/ConsequencesofElevated HbA1c in type2 Diabetes/a/32729.NYU school of medicine.
20. Walker D, Carrington A, Cannan S et al. Structural abnormalities do not explain the early functional abnormalities in the pe-

 https://doi.org/10.46389/rjd-2020-1005 © 2020 The Authors

ripheral nerves of the streptozotocin diabetic rat. J anat 195(Pt 3):419-427, 1999.

21. Greene DA, Arezzo JC, Brown MB and the Zenarestat study Group. Effect of aldose reductase inhibition on nerve conduction and morphometry in diabetic neuropathy. Neurology 53(3), 580–580, 1999.
22. Börü ÜT, Alp R, Sargin H et al. Prevalence of peripheral neuropathy in type 2 diabetic patients attending a diabetes center in Turkey. Endocrine journal. 51(6): 563-7, 2004.
23. Ugoya SO, Puepet FH, Ugoya TA, Agaba E. Risk determinants of diabetic peripheral neuropathy in Jos, North-Central Nigeria. J of Chinese Clinical Medicine 3(5): 285-91, 2008.
24. Nicholson G. Penetrance of the hereditary motor and sensory neuropathy la mutation: Assessment by nerve conduction studies. Neurology 41(4): 547-547, 1991.
25. Trivedi JR, Phillips L, Chhabra A. Hereditary and acquired polyneuropathy conditions of the peripheral nerves: clinical considerations and MR neurography imaging. Seminars in musculoskeletal radiology. 2015.
26. Tavakkoly-Bazzaz J, Amoli MM, Parvica V et al. VEGF gene polymorphism association with diabetic neuropathy. Molecular biology reports 37(7): 3625-3630, 2010. [in persian]
27. Freedman M, Gehert JA, Young G, Kamen L. Challenging Neuropathic Pain Syndromes: Evaluation and Evidence-Based Treatment. Elsevier (ed), 3251 Riverport lane, 2018
28. Schumacher M, Hussain R, Gago N et al. Progesterone synthesis in the nervous system: implications for myelination and myelin repair. Frontiers in neuroscience 6: 10, 2012.
29. Kleinbaum DG, KleinM. Survival analysis: a self-learning text. 3rd ed. Statistics for biology and health 2012.

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Original Article

Evaluation of Unstimulated Saliva Levels in Controlled and Uncontrolled Type 2 Diabetes Mellitus

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Abstract

Introduction: Diabetes is a common syndrome characterized by a steady rise in blood glucose levels and has various side effects, including oral complications such as periodontitis and dry mouth. **Material and Methods:** This case-control study was performed on 30 patients with type 2 diabetes mellitus and 30 normal people who were referred to Kowsar Hospital of Semnan, Iran. The study groups were evaluated for HbA1C, and unstimulated saliva and data were statistically analyzed. **Results:** Unstimulated saliva in diabetic patients was 0.02 ± 0.07 ml/ min and ± 14.0 ± 02.0 ml in the control group (P <0.001). In the diabetic group, 18 out of the 30 diabetic patients (60%) had uncontrolled glucose (HbA1c was 7% or higher), and the rest had controlled glucose. According to the HbA1c study, the mean standard deviation of unstimulated saliva in controlled diabetic patients was 0.08 0 ± 0.02 ml/min and 0.06 ± 0.02 ml/min in uncontrolled patients, which showed a significant difference (p=0.006). **Conclusion:** Dry mouth was more prevalent in people with uncontrolled diabetes, so blood glucose level and its control had a significant effect on the reduction of unstimulated salivation.

Keywords: Diabetes Mellitus, Type 2 Diabetes, Saliva, Unstimulated Saliva, HbA1C.

Introduction

Diabetes is a syndrome caused by abnormal metabolism of fats, proteins, and carbohydrates, which causes a partial or complete deficiency of insulin in the body, which is characterized by elevated blood glucose levels. Another disease type is caused by increased cellular insulin resistance. A third form occurs in pregnant women who show abnormal adaptation to glucose [1, 2].

The prevalence of the disease in 2010 was around 221 million people worldwide, which is 2 or 3 times higher in some areas of the world, such as Africa and Asia. Diabetes, especially its second type, has become increasingly prevalent in developing countries for various reasons such as population growth, age, inactivity, and unhealthy diet. These people are often middle-aged (35-64 years) and have a high-income. In developed countries, however, type 2 diabetes usually occurs more often in older and retired people [2, 3].

Diabetes has many effects that can affect various organs of the body, including the mouth. Numerous complications of diabetes may involve the mouth, such as periodontitis and xerostomia [3]. Diabetes with poor glucose control leads to the destruction of salivary

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glands and consequently to xerostomia [4], which may cause many complications including irritation of the dry mucosa, minor ulcerations, microorganisms overgrowth for opportunistic fungi like Candida, and increased gum fluid glucose, expanded dental caries and heightened plaque accumulation [1, 5].

Xerostomia is an uncomfortable complication of diabetes that can decrease a person's quality of life. It is more associated with poor glycemic control and also the medications which deactivate salivary glands rather than diabetes itself. Autonomic system neuropathy is another complication of diabetes that affects and decreases salivary secretion, as the salivary flow is controlled by sympathetic and parasympathetic pathways [1, 5].

Diabetes has many oral manifestations, such as burning mouth, changing in wound healing, xerostomia, increased incidence of infections, candidiasis (especially pseudomembranous acute candidiasis of the tongue, buccal mucosa, and gingiva) and bilateral enlargement of the salivary glands. It is also a proven risk factor for the prevalence and severity of gingivitis and periodontitis [1, 6]. Xerostomia is more likely to be the result of medications taken by a diabetic, not diabetes itself. Dry mucosal surfaces that are caused by a decrease in salivary secretion are easily irritated and lead to minor mucosal ulcers, a burning sensation in the mouth, and an increased likelihood of fungal infections.

The study of Harijanti k. et al. from 2007 showed that 76% of type II diabetic patients have xerostomia [7] and Andrades k. et al. showed in 2011 that the prevalence of the xerostomia in type I diabetic patients was 64% [8]. Abdullah M et al. declared in 2015 that the prevalence of xerostomia was 16.07% in all individuals [9].

There have been numerous studies on the complications of diabetes, but none of the previous studies evaluated the xerostomia in diabetic people from Semnan. In addition, this study will measure the effect of glycemic control on the unstimulated saliva. Due to the above-mentioned problems of xerostomia, we decided to assess the extent of this complication in order to plan, if necessary, to improve the treatment and reduce these significant complications in diabetic patients and cure xerostomia by controlling diabetes.

Material and Methods

Study design and patients

This case-control study was performed by measuring unstimulated saliva using a graduated cylinder. Thirty diabetic patients and 30 healthy patients that were referred to Kosar hospital in Semnan during 2018, were selected by continuous sampling. The subjects were considered diabetic based on fasting blood sugar (FBS) levels of 126 mg/100ml or higher. The included patients fit in the 20-60 age category with the above-mentioned FBS level who signed an informed consent form, and exclusion criteria consisted of those who received antidepressants, antihistamines, decongestants, muscle relaxants and antihypertensives; individuals under chemotherapy and radiotherapy; a history of nerve damage due to head and face injuries, smoking and tobacco use, autoimmune diseases such as Sjögren's disease, AIDS and others; methamphetamine consumption and oral conditions that cause xerostomia irrelevant to diabetes.

Laboratory, anthropometric and clinical data collection

Unstimulated saliva was collected by the spitting method, in which the patients were asked to collect saliva in their mouth, then spit in a pre-weighed tube, every 60 seconds, do this for 5 minutes, then continue for 15 minutes. The confounding variables were identified. Data such as diabetes type, duration of illness, and the latest HbA1c test were also recorded in this questionnaire.

Statistical analysis

Data were then gathered in the form of data collection and analyzed using Shapiro Wilk, Chi-square, one-way ANOVA (t-student analysis), and Tukey's test in linear regression analysis and SPSS 23.0 with a 5% significance level; then, statistical analysis was performed.

Ethical approval

This study was approved by the Ethics Committee of the Semnan University of Medical Sciences (No. IR.SEMUMS.REC.1397.084).

Results

In this study, 30 patients with diabetes and 30 healthy people were evaluated, and the results were as follows:

46.7% (n = 14) of patients from the diabetic and control groups were female, and the sex distribution of the

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Table 1: Mean and standard deviation of unstimulated saliva levels in patients with controlled and uncontrolled diabetes.

	HbA1c	Percentage	Number	Average	Standard deviation	p-value
Controlled diabetes	7>	40	12	0.08	0.02	0.006
Uncontrolled Diabetes	7 ≤	60	18	0.06	0.02	0.006

Table 2: The results of regression analysis on factors associated with unstimulated saliva levels in diabetic patients.

Variable	Regression coefficient (β)	The standard error	p-value
HbA1C	-0.039	0.006	<0.001
Constant coefficient	0.346	0.047	<0.001

two groups was homogeneous (P = 0.956). Mean ± SD of the age of the diabetic patients was 41.3 ± 12.0 years, and 42.9 ± 12.0 years for the control group; the difference was not significant (P = 0/541).

The mean ± SD of unstimulated saliva in the control group was 0.14 ± 0.02 ml/min.

Of the 30 diabetic patients, 60% (n = 18) had uncontrolled glucose levels (HbA1c was 7% or higher), and the rest had uncontrolled glucose levels. Mean ± SD of unstimulated saliva in controlled diabetic patients was 0.08 ± 0.02 ml/min and 0.06 ± 0.02 ml/min in uncontrolled diabetic patients; there was a significant difference (p = 0.006) (Table 1).

Linear regression analysis showed that only HbA1c had a negative and significant correlation with unstimulated saliva in diabetic patients (Table 2).

Discussion

The Mean ± SD values of unstimulated saliva in pre-diabetic patients was 0.12 ± 0.01 ml/min and 0.14 ±0.02 ml/min in the control group, which was statistically significant (p <0.001). Because this was the first time that unstimulated saliva was measured in pre-diabetic patients, no similar study was found based on our database search.

The mean ± SD values of unstimulated saliva in controlled and uncontrolled diabetic patients were 0.08 ± 0.02 ml/min and 0.06 ± 0.02 ml/min, respectively (p <0.006).

HbA1C shows the average blood sugar level in the last one to three months. When HbA1C levels were higher than 7%, the patients were considered as uncontrolled diabetics. Regarding the comparison between unstimulated saliva in controlled and uncontrolled patients in this study, decreased unstimulated saliva and increased xerostomia were demonstrated in uncontrolled diabetic patients.

Investigation of the relationship between unstimulated saliva content with HbA1C in order to determine long-term metabolic control of glucose despite multiple searches in all databases was unsuccessful, and the results were inconsistent.

Chavez EM et al. concluded in 2000 and 2001 that diabetic glucose control had an effect on salivation and decreased salivary HbA1C levels [10, 11]. However, regarding the relationship between salivary HbA1C levels and FBS, Andrades K. et al. showed in 2011 that the accuracy of salivary levels is higher than glycemic control, which may be due to the absence of a control group, which is critical in these studies [12].

Siudikiene et al. [13], Busato et al. [14], and Bernardi et al. [15] concluded that glycemic control does not affect the salivary flow rate, contrary to the results we have obtained in this study, which may be due to various reasons. For instance, Siudikiene et al. considered uncontrolled sugar levels above 9% [13], and Busato et al. had a small sample size, did not use a control group, and also opted for an 8% determination to indicate controlled and uncontrolled sugar levels [14], as well as in the Bernardi's et al. study [15].

As Ahadian et al. mentioned, xerostomia and a low unstimulated whole saliva (UWS) flow were more common in DM patients than controls; in addition, they proved that adequate glycemic control could prevent xerostomia, results which are consistent with those of the present study. [16]

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There are numerous oral manifestations in diabetic patients, many of which depend on the degree of glycemic control. Mucosal diseases include oral sensory disturbances such as a burning sensation of the mouth, changes in wound healing, xerostomia, increased incidence of infections (especially acute candidiasis of the tongue, buccal and gingival mucosa) [1, 17-20]. Also, diabetes mellitus is the proven risk factor of the prevalence and severity of gingivitis and periodontitis [20].

Pathophysiologically, in diabetes, xerostomia is a result of bilateral enlargement of the salivary glands or sialadenitis (especially regarding the parotid glands); the effects of anti-glycemic drugs, polyuria, and salivary gland neuropathy may be frequently associated with glycemic control [1]. Therefore, timely diagnosis of this complication, such as delay wound healing post-extraction [21] in pre-diabetic patients, can prevent further difficulties.

There were limitations in this study, such as the quality and chemistry of saliva, as well as the lack of cooperation of the patients that caused the specimens to shed, and more samples were taken to resolve these problems in order to provide a specific sample size.

Conclusion

Dry mouth was more prevalent in people with uncontrolled diabetes, so blood glucose level and its control had a significant effect on the reduction of unstimulated salivation.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Lamster IB, Lalla E, Borgnakke WS et al. The relationship between oral health and diabetes mellitus. J Am Dent Assoc 139: 19S-24S, 2008.
2. Ship JA. Diabetes and oral health: an overview. J Am Dent Assoc 134: 4S-10S, 2003.
3. Rabiei MA, Mohtasham Amiri Z, Kalantari SA et al. Oral Soft Tissue Pathologies among Diabetic Patients in Rasht-2005. SSU_Journals 15(3): 46-52, 2007.
4. Malicka B, Kaczmarek U, Skośkiewicz-Malinowska K. Prevalence of xerostomia and the salivary flow rate in diabetic patients. ADV CLIN EXP MED 23(2): 225-33, 2014.
5. Rostaeizade Z. Reliability of the sialomy method in patients with oral dry mouth and control group: J Kerman Univ Med Sci 9: 221-230, 2014.
6. Moore PA, Guggenheimer J, Etzel KR et al. Type 1 diabetes mellitus, xerostomia, and salivary flow rates. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 92(3): 281-291, 2001.
7. Harijanti K, Soebadi B, Mulyaningsih I. Prevalence of xerostomia on type 2 diabetes mellitus in Hajj Hospital Surabaya. Dent. J. (Majalah Kedokteran Gigi) 40(3): 136-139, 2007.
8. Andrades KM, Oliveira GB, Avila LD et al. Association of glycemic indexes, hyposalivation, and xerostomia type 1 diabetic patients. Int J Odontostomat 5(2): 185-190, 2011.
9. Abdullah MJ. Prevalence of xerostomia in patients attending Shorish dental speciality in Sulaimani city. Journal of clinical and experimental dentistry 2015; 7: e45.
10. Chavez EM, Taylor GW, Borrell LN et al. Salivary function and glycemic control in older persons with diabetes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 89(3): 305-311, 2000.
11. Chávez EM, Borrell LN, Taylor GW et al. A longitudinal analysis of salivary flow in control subjects and older adults with type 2 diabetes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 91(2):166-173, 2001.
12. Andrades KM, Oliveira GB, Avila LD et al. Association of glycemic indexes, hyposalivation, and xerostomia type 1 diabetic patients. Int J Odontostomat 5(2): 185-190, 2011.
13. Siudikiene J, Machiulskiene V, Nyvad B et al. Dental caries and salivary status in children with type 1 diabetes mellitus, related to the metabolic control of the disease. Eur J Oral Sci 114(1): 8-14, 2006.
14. Busato IM, Ignácio SA, Brancher JA et al. Impact of xerostomia on the quality of life of adolescents with type 1 diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108(3): 376-382, 2009.
15. Bernardi MJ, Reis A, Loguercio AD et al. Study of the buffering capacity, pH and salivary flow rate in type 2 well-controlled and poorly controlled diabetic patients. Oral Health Prev Dent 5(1): 73-78, 2007.
16. Ahadian H, Akhavan Karbassi MH, Afkhami-Ardekani M et al. Comparison of the Unstimulated whole Saliva Flow Rate in Diabetic Type II Patients with Healthy Individuals. IJDO 6(2): 93-97, 2014.
17. Ghadiri-Anari A, Hazar N, Sadrabad MJ et al. Comparing the frequency of some oral lesions in prediabetic and healthy individuals: Is there any difference?. Int J Prev Med 10(1): 177, 2019.
18. Ghadiri-Anari A, Kheirollahi K, Hazar N et al. Prevalence of risk factors in diabetic patients with oral complications. Koomesh 21(3): 477-485,2019.

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19. Ghadiri-Anari A, Namiranian N, Hazar N et al. Relationship Between Oral Presentations and Diabetic Micro-Vascular Complications. Shiraz E Med J Online ahead of Print 20(4): e80081, 2018.
20. Burket LW, Greenberg MS, Glick M. Burket's oral medicine: diagnosis and treatment. BC Decker; 2003.
21. Karbassi MH, Salehi R, Kheirollahi K et al. The relationship between socket blood sugar and post-extraction complications in type II diabetic and non-diabetic patients. IDO 7(1): 13, 2015.

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Original Article

SIRT1 Level Differences among Old Rats, Streptozotocin-Induced Diabetic Rats, and D-Galactose-Induced Aging Rats

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Abstract

Introduction: The Sirtuin 1 (SIRT1) level is suspected to be lower in older ages. In a study of rats, aging can be induced by D-galactose. Various degenerative diseases can also develop from streptozotocin induction (rat models for diabetes mellitus). This study aimed to verify SIRT1 level differences among old rats, D-galactose-induced diabetic rats and streptozotocin-induced aging rats. **Material and Methods:** A total of 20 female Wistar rats aged four months was divided into five groups of 4 rats each. Group K1 was directly terminated, K2 and K3 were kept until 18 and 33 months old, respectively, and later terminated. Rats in the K4 group were induced with streptozotocin at a dose of 65 mg/kg BW and nicotinamide at 120 mg/kg BW and kept for one month. Rats in the K5 group were induced by 300 mg/kg BW of D-galactose for one month. Blood specimens were taken to examine SIRT1 levels using ELISA. The statistical test used in this study was the Kruskal-Wallis test (95% confidence level). **Results:** SIRT1 levels in rats aged 4, 18, and 33 months, diabetic rat models, and D-galactose-induced aging were 0.43 ± 0.12, 0.58 ± 0.15, 0.30 ± 0.01, 0.25 ± 0.13, and 0.21 ± 0.05, respectively (p=0.028). **Conclusions:** D-galactose-induced aging rat models had the lowest SIRT1 level compared to 33-month-old rats and diabetic rat models.

Keywords: Aging, D-galactose, rat model, SIRT1, streptozotocin.

Introduction

The number of older people continues to increase in Indonesia as well as worldwide [1]. However, this condition reduces physiological functions and is commonly associated with the increasing prevalence of degenerative diseases [2]. To overcome various problems among the elderly and to improve their quality of life, different studies related to old age and its associated diseases are required.

Research involving humans requires an in-depth ethical review; consequently, studies involving experimental animals will be more frequently conducted. Animal models for old age are important and developed based on the pathophysiological process of aging [3]. The aging rat modeling is widely used because of its affordability and the easiness of D-galactose application. According to one of the theories of aging, the administration of D-galactose can increase reactive oxygen species (ROS), leading to the death of a large number of cells [4, 5]. However, the use of D-galactose in rats has yet to be effective [6]. Therefore, the parameters of the aging marker in D-galactose models should be proven.

Diabetic rats through streptozotocin induction can also be used as an aging animal model as it can lead to degenerative diseases, such as Alzheimer's disease [7], through the mechanism of telomere instability [8], which is in accordance with one of the aging mechanisms where telomere shortening occurs and ultimately causes cell apoptosis [9].

Aging animal models should be justified by the presence of aging markers. However, due to the complexity of the aging mechanism, no single valid marker is available [10], but one secure method to achieve is by assessing the markers in a peripheral blood specimen. For example, Rusanova et al. used plasma advanced

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oxidation protein products (AOPP) and lipid oxidation (LPO) in their study [11]. Both markers indicate an increased level of ROS [12]. Aging has been recently associated with some metabolic disorders and leads to dyslipidemia and increased levels of triacylglycerol/TAG [13], triglycerides (TG), and low-density lipoprotein (LDL) as well as an increasing atherogenic index (AI) during the aging process [14]. However, the upstream of lipoprotein regulation contains Sirtuin 1 (SIRT1) [15].

SIRT1 is the most recent marker associated with the pathogenesis of aging [14, 16] and is a deacetylation enzyme [17]. The mechanism of SIRT1 deacetylation is associated with protection against the process of cell aging. SIRT1 also plays an essential role in cell cycle regulation, energy homeostasis, apoptosis, circadian rhythm, mitochondrial function, as well as lipid and glucose metabolism [18]. SIRT1 maintains the physiology of young age and extends lifespan [19]. SIRT1 also stimulates the sympathetic nervous system, maintains the morphology and mitochondrial function of skeletal muscles for physical activities, and also regulates body temperature and oxygen consumption during aging. Previous studies have identified the role of SIRT1 as an aging marker. SIRT1 decreases in Alzheimer's and mild cognitive impairment (MCI) conditions. Sirtuin levels are also lower in older people compared to young people [20]. Therefore, this study aims to determine the differences in SIRT1 blood levels among old-aged rats, diabetic rats (induced by streptozotocin and nicotinamide), and aging rats (induced by D-galactose).

Material and Methods

Study design and patients

This research was an experimental study with female Wistar rats, and the study was conducted from March 2018 to September 2019.

Research Subjects

The rats were obtained from the Physiology Laboratory of the Faculty of Medicine, Universitas Islam Indonesia and taken care of at the Research Laboratory of the Faculty of Medicine, Universitas Islam Indonesia. All of the rats came from the same dam, and a total of 16 rats aged 4 months and 4 rats aged 18 months were involved in the study. The rats were randomized, except the 18-month-old rats, and divided into five groups, each with four rats. The K1 and K2 groups consisted of 4-month-old rats, which were kept until termination at 4 months (K1) and 18 months (K2). The rats aged 18 months were kept and terminated at 33 months old (K3), while the K4 group with 4-month-old rats had diabetes mellitus (DM) induced with 65mg/kg BW streptozotocin and 120mg/kg BW nicotinamide through intraperitoneal injections, maintained for a month post-induction. The K5 group comprised a group of rats induced by 300mg/kg BW of D-galactose through daily intraperitoneal injection for one month and were then terminated.

Determination of Sample Size

The number of experimental animals used in this study was determined using the formula [21] E= total number of animals - total number of groups = 10 - 20. E is a constant within a range of 10-20. Based on the calculation, the total number of animals that should be used in this study was between 15-25 rats, with each group consisting of 3-5 rats. It was then decided for this study that the number of experimental animals used in each group should be four rats.

Treatment

No specific treatment was given to the experimental animals, and they were kept up to a specified age. During the treatment, the rats were given ad libitum food and drink, treated in a group cage, and provided with a light-dark cycle. Two rats of group 4 were tested for the blood glucose levels before, after three days, and after one month of streptozotocin and nicotinamide induction. Meanwhile, the rats in group 5 were examined for malondialdehyde (MDA) levels before and after the induction with D-galactose.

Measurement and Laboratory Analysis

On the predetermined termination day, the rats were anesthetized with ketamine injected intramuscularly. After they were unconscious, 2 ml of blood was taken through the heart. Each blood specimen was inserted into an Eppendorf tube to be centrifuged to obtain blood plasma.

Assessment of blood glucose levels was carried out using the rapid test method, whereas the MDA level was examined at the Food and Nutrition Laboratory of PAU UGM using the Thiobarbituric Acid Reactive Substance (TBARS) assay.

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ELISA

The blood specimens centrifuged at 3000 g for 15 minutes were stored at -80°C before the ELISA test was performed. A microplate was coated with the same amount of serum specimen in each well, and the standard protocol for SIRT1 testing using the FineTest ELISA kit was followed. The results were read on the ELISA reader at a wavelength of 450. Eight different concentrations of purified SIRT (0, 1.562, 3.125, 6.25, 12.5, 25, 50, and 100 ng/ml) were used to prepare the standard curve. The SIRT1 level was calculated using the formula y = 0.4492x2 + 0.4795x + 0.0244, R2 = 0.983.

Statistical analysis

The Kruskal-Wallis test was used to determine the significant differences in mean SIRT1 levels across the groups. The differences in every two groups were examined using the Independent t-test for normally distributed data and the Mann-Whitney U test for the remaining data.

Ethical approval

The treatment of experimental animals used in this study was carried out by following animal welfare standards. This research protocol was approved by the Ethics Committee of the Faculty of Medicine, Universitas Islam Indonesia (No. 85/Ka.Kom.Et/70/KE/IV/2018).

Results

Three days after induction, the blood glucose level showed an increase (pre: 166.25 mg/dl, post: 216.25 mg/dl), indicating that the rats had DM since the blood glucose level exceeded 200mg/dl. The rats also experienced polydipsia, polyphagia, and polyuria. Prior to being terminated in the fourth week, the blood glucose level remained at > 200 mg/dl (310.25 mg/dl), while in group 5, the MDA level increased (pre: 1.08 nmol/ml and post: 9.35 nmol/ml).

The highest body weight at termination was found in the 18-month age group, and the lowest was in the 4-month age group. The rats' body weight decreased at old age (33 months) and after induction with streptozotocin and D-galactose (Table 1). The SIRT1 level was the highest at 18 months old and the lowest among the aging model rats induced by D-galactose (Figure 1).

Table 1: Body weight of the rats at termination.

Group (termination age)	Weight of the rat Mean ± SD
K1 (4 month)	118 ± 6.78
K2 (18 months)	158 ± 9.09
K3 (33 months)	140 ± 8.16
K4 (5 months)	121 ± 10.30
K5 (5 months)	120.75 ± 2.98

Note: Termination age of K1: 4 months, K2: 18 months, K3: 33 months, K4: 5 months, K5: 5 months.

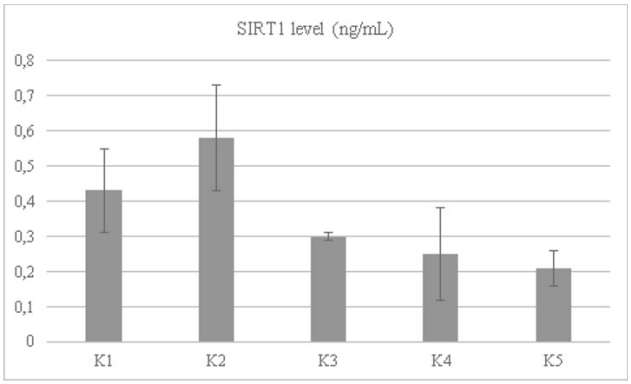

Figure 1: SIRT1 levels in the young age, old age, and aging models (p value=0.028). Note: Termination age of K1: 4 months, K2: 18 months, K3: 33 months, K4: Induced DM, K5: D-galactose-induced.

The post-hoc test showed that the level of SIRT1 in the D-galactose group was significantly lower compared to the age group of young rats) and even lower (significant) in old-aged rats (33 months). The DM model mice also had low SIRT1 levels and were not different from the D-galactose group or the old aged rat group (Table 2).

Table 2: The differences in SIRT1 levels between old aged, induced DM and D-galactose-induced rats.

Group	p value			
	K2	K3	K4	K5
K1	0.16	0.12	0.25	0.017
K2	-	0.032	0.08	0.004
K3	-	-	0.25	0.017
K4	-	-	-	0.386

Note: K: 4 months, K2: 18 months, K3: 33 months, K4: induced DM, K5: D-galactose-induced.

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Discussion

This study shows that the blood levels of SIRT1 change due to aging. There was a pattern of increasing SIRT 1 levels among rats aged 18 months compared to those aged 4 months, whereas a decrease occurred at the age of 33 months. In addition, there were decreased SIRT1 levels in rats wit DM (group 4) and at a high level of ROS in the body of rats from group 5. Of the five study groups, the highest SIRT1 levels were found in the 18-month age group, and the differences in SIRT1 levels among the five groups in this study were statistically significant. Significant differences were found among 18-month-old rats compared to the 33-month-old rats and among the high ROS-induced model group (group 5) compared to the 4-month, 18-month, and 33-month rat groups (Table 2). This shows that changes in SIRT1 levels are mainly influenced by the changes in age and ROS levels in the blood. In the DM group, there was a decrease in SIRT1 levels slightly above the D-galactose group, but no significant difference was found.

The decreased SIRT1 levels in this study are in line with the study of Lee and Yang [14], which suggests that decreasing SIRT1 levels can be found after the age of 40 years in women and after 50 years in men. There is no significant difference in SIRT1 levels between men and women. Aging also changes SIRT1 expression in body tissues as proven using immunohistochemistry, which shows that despite an increase in SIRT1 expression in the lungs and skin of 24-month-old rats, such expression decreases in the kidney and spleen [22]. This indicates that changes in the pattern of SIRT1 levels exist not only in the blood (systemic changes) but also in the tissues, and such changes are tissue-specific.

SIRT1 levels are evident in aging rat models. In aged animal models with genetic modification of senescence-accelerated mouse prone (a lifespan of 10-17 months) and senescence-accelerated mouse resistant 1 (a lifespan of 19-21 months), it was found that aging results in decreased SIRT1 levels in the white adipose tissue, brain, liver, and muscles. This is observable at both the transcription level and the translation level. Also, the lowest levels are found in the brain [23]. The results of this study indicate that the lowest decrease in SIRT1 serum levels is found in aging rat models (induction of increased ROS through D-galactose administration), and it is in line with the study of Gong et al. [23].

This study also shows that metabolic conditions also influence the decrease in SIRT1 levels due to a high blood glucose level. This is similar to the study by De Kreutzenberg et al., which found that insulin resistance and metabolic disorders correlate with the low SIRT1 gene expression in peripheral blood mononuclear cells [24]. SIRT1 negatively correlates with the index for subclinical atherosclerosis, as low levels of SIRT1 are pro-atherogenic by nature. Decreased SIRT1 can also be found in pre-diabetic conditions, indicating that SIRT1 acts as a negative metabolic signal transducer. High glucose levels in the body result in low levels of nicotinamide adenine dinucleotide (NAD+). Because SIRT1 is an NAD+-dependent enzyme, a decline in this compound will result in low SIRT1 activities [24].

Aging will also cause mitochondrial dysfunction in muscle cells, adipocytes, and monocytes/macrophages as one of the pathogeneses of insulin resistance and type 2 DM in aging. Such conditions will result in the activation of monocytes in the circulation, as well as adipocytes and macrophage cells in fat tissues to induce an inflammatory process. Eventually, tumor necrosis factor (TNF)-α, Interleukin-6, and monocyte chemoattractant protein-1 (MCP-1) cytokine release will activate the inhibitor kappaB kinase (IKK) and c-Jun N-terminal kinases (JNK) inflammatory pathways. Existing hyperglycemia, inflammation, and hypercholesterolemia will also result in increased production of ROS by mitochondria [25]. The diabetic rat models seem to be able to model aging because of the low SIRT1 levels.

In the D-galactose group, SIRT1 levels reached the lowest values because of increased ROS levels. SIRT1 levels decrease extremely significantly when exposed to hydrogen peroxidase/H2O2 [26]. In contrast, SIRT1 plays an essential role as a longevity factor and regulates autophagy and ROS production. High levels of ROS will inhibit SIRT1 activities by influencing its cysteine residues. High levels of ROS also increase cell inflammation [27]. On the other hand, overexpression of SIRT1 will result in milder inflammation [18] and inhibit nuclear factor-kappa B (NFKB), one of the pro-inflammatory cytokines [19]. The increase in such cytokines will raise the population of pro-inflammatory/M1 macrophages and oppositely reduce anti-inflammatory/M2 macrophages [25].

SIRT1 assists genetic stability in an oxidative stress condition made possible by SIRT1 deacetylation in the genes that play a role in DNA repair. SIRT1 will also reduce Bcl2 pro-apoptotic protein expression and prevent Caspase-3 breakdown [26]. Such a mechanism will eventually prevent early cell death. SIRT1 overexpression will improve the production of enzymes that play an essential role as antioxidants. One of the mechanisms of aging is increased apoptosis, while the SIRT1 mechanism of action occurs through stress resistance

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and prevention of cell apoptosis due to SIRT1 deacetylation against p53, Foxo, and Ku79. The interaction of SIRT1 with Ku79 increases the sequestering of the Bax protein and also improves DNA repair activities [18].

This study has some limitations in that no further examination was conducted on other parameters (including levels of ROS, antioxidants, cholesterol, and others) that can support the mechanism of action of SIRT1 in the body. Therefore, the correlation between these parameters and changes in SIRT1 levels in the blood of aging animals or animals with certain metabolic disorders remains unknown. This study did not observe the pattern of changes in SIRT1 expression in body tissues either. Therefore, further research is expected in order to identify the mechanism of action of SIRT1 in an aging process and metabolic disorders.

Conclusions

This research has proved that there was a change in the pattern of SIRT1 levels in the blood of aging animal models. The aging model using D-galactose indicated the existence of aging markers in the form of low SIRT1 levels and was proven to be more useful than waiting and caring for rats until old age. The streptozotocin model, although not as good as D-galactose, also showed signs of aging due to lower levels of S1RT1 compared to older rats and indicated no differences compared to the D-galactose rat models. Detection of SIRT1 blood levels can be an alternative to observe metabolic disorder risks, particularly those related to increased ROS levels.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Kementrian Kesehatan RI. Gambaran Kesehatan lanjut Usia di Indonesia. Bul Jendela Data dan Inf Kesehat Semester I::1-18, 2013.
2. National Institute on Aging, National Institutes of Health. World Global Health and Aging. US Department of Health and Human Services. World Health Organization.; 2011.
3. Mitchell SJ, Scheibye-Knudsen M, Longo DL, de Cabo R. Animal Models of Aging Research: Implications for Human Aging and Age-Related Diseases. Annu Rev Anim Biosci 3: 283–303, 2015.
4. Hadzi-petrushev N, Stojkovski V, Mitrov D, Mladenov M. D-Galactose induced changes in Enzymatic Antioxidant Status in Rats of Different Ages. Physiol Res 64(1): 61–70, 2015.
5. Cui H, Kong Y, Zhang H. Oxidative Stress, Mitochondrial Dysfunction, and Aging. J Signal Transduct 2012: 1–13, 2012.
6. Parameshwaran K, Irwin MH, Steliou K, Pinkert CA. d-Galactose Effectiveness in Modeling Aging and Therapeutic Antioxidant Treatment in Mice. Rejuvenation Res 13(6): 729–736, 2010.
7. Paviolo NS, Santiñaque FF, Castrogiovanni DC, Folle GA, Bolzán AD. The methylating agent streptozotocin induces persistent telomere dysfunction in mammalian cells. Mutat Res Genet Toxicol Environ Mutagen 794: 17–24, 2015.
8. Kamat PK. Streptozotocin induced Alzheimer's disease like changes and the underlying neural degeneration and regeneration mechanism. Neural Regen Res 10(7): 1050–1052, 2015.
9. Park DC, Yeo SG. Aging. Korean J Audiol 17: 39–44, 2013.
10. Wagner K, Cameron-smith D, Wessner B, Franzke B. Biomarkers of Aging : From Function to Molecular Biology. Nutrients 8: 1–12, 2016.
11. Rusanova I, Diaz-Casado ME, Fernández-Ortiz M et al. Analysis of Plasma MicroRNAs as Predictors and Biomarkers of Aging and Frailty in Humans. Oxid Med Cell Longev 2018: 7671850, 2018.
12. Razygraev A V, Petrosyan MA, Tumasova ZN et al. Activity of glutathione peroxidase in rat blood plasma and serum: Postnatal and aging-associated alterations. Adv Gerontol (Uspekhi Gerontol) 32: 38–44, 2019.
13. Kawanishi N, Kato Y, Yokozeki K et al. Effects of aging on serum levels of lipid molecular species as determined by lipidomics analysis in Japanese men and women. Lipids Health Dis 17: 1–8, 2018.
14. Lee HJ, Yang SJ. Aging-Related Correlation between Serum Sirtuin 1 Activities and Basal Metabolic Rate in Women, but not in Men. Clin Nutr Res 6(1): 18, 2017.
15. Ye X, Li M, Hou T, et al. Sirtuins in glucose and lipid metabolism. Oncotarget 8(1): 1845–1859, 2017.
16. Turkmen K. Sirtuins as novel players in the pathogenesis of diabetes mellitus. World J Diabetes 5: 894, 2014.
17. Stacchiotti A, Favero G, Rezzani R. Resveratrol and SIRT1 Activators for the Treatment of Aging and Age-Related Diseases. In: Resveratrol - Adding Life to Years, Not Adding Years to Life IntechOpen, 2019
18. Nogueiras R, Habegger KM, Chaudhary N et al. Sirtuin 1 and sirtuin 3: Physiological modulators of metabolism. Physiol Rev 92: 1479–1514, 2012.
19. Satoh A, Brace CS, Rensing N et al. Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell Metab 18: 416–430, 2013.
20. Kumar R, Chaterjee P, Sharma PK et al. Sirtuin1 : A Promising Serum Protein Marker for Early Detection of Alzheimer ' s Disease. PLoS One 8: e61560, 2013.

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21. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian J Psychol Med 35: 121, 2013.
22. Kwon Y, Kim J, Lee CY, Kim H. Expression of SIRT1 and SIRT3 varies according to age in mice. Anat Cell Biol 48: 54–61, 2015.
23. Gong H, Pang J, Han Y et al. Age-dependent tissue expression patterns of Sirt1 in senescence-accelerated mice. Mol Med Rep 10: 3296–3302, 2014.
24. De Kreutzenberg SV, Ceolotto G, Papparella I et al. Downregulation of the longevity-associated protein sirtuin 1 in insulin resistance and metabolic syndrome: Potential biochemical mechanisms. Diabetes 59: 1006–1015, 2010.
25. Kitada M, Ogura Y, Monno I, Koya D. Sirtuins and type 2 diabetes: Role in inflammation, oxidative stress, and mitochondrial function. Front Endocrinol (Lausanne) 10: 1–12, 2019.
26. Chen T, Dai SH, Li X et al. Sirt1-Sirt3 axis regulates human blood-brain barrier permeability in response to ischemia. Redox Biol 14: 229–236, 2018.
27. Salminen A, Kaarniranta K, Kauppinen A. Crosstalk between oxidative stress and SIRT1: Impact on the aging process. Int J Mol Sci 14(2): 3834–3859, 2013.

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Original Article

The Remodeling of Lungs Under the Influence of Alloxan-Induced Hyperglycemia

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Abstract

Introduction: Diabetes mellitus is a global medical and social problem. 2-3% of the population suffers from diabetes mellitus. According to current forecasts, the number of people with diabetes mellitus will double every 15 years. Diabetes mellitus is the basis for the development of complex comorbidities and complications such as diabetic microangiopathy and neuropathy. Diabetes patients are at increased risk for atherosclerosis, coronary heart disease, and renal failure. Scientists around the world have been thoroughly engaged in the problem of hyperglycemia, which is the consequence of type 1 diabetes mellitus, but the causes of its occurrence have not been fully established yet. The study aimed to investigate the lungs of pre-reproductive age rats under the influence of alloxan-induced hyperglycemia. **Material and Methods:** 12 rats of 3 months of age were involved in the experiment. They were divided into two series: experimental and control. The Control series includes six intact rats. For the experimental modeling of hyperglycemia, we used alloxan monohydrate, pre-dissolved in 0.9% solution of sodium chloride, which was then injected intraperitoneally once at a dose of 20 mg/100 g on an empty stomach. The experiment lasted 60 days. We measured the glucose level using the Accu-Chek Advantage (Boehringer, Germany) at2, 12, and 24 hours after alloxan injection, and then weekly. The average level of glucose in the blood remained at 11 mmol/L ± 2 mmol/L.
To study the remodeling of the lungs, we used the histological examination by a light microscope (Olympus BH-2) and histomorphometry using the universal certified program "SEO Scan Lab 2.0" and "SEO Image Lab 2.0". The obtained data were processed on a personal computer using the software "GraphPad" and a licensed Excel XP spreadsheet editor (Microsoft Office 2013). The data were analyzed by unpaired t-test. P values ≤0.05 were considered as statistically significant. **Results:** Obtained morphometric data shows the increase of the width of alveoli by 8.5% ($p < 0.05$), the width of the conducting part of the respiratory bronchiole by 15.7 % ($p < 0.05$) and the thickness of the interalveolar septum by 8.3% ($p < 0.05$). The wall thickness of pulmonary vessels increased by 22.9% ($p <0.05$). Lymphoid nodules were more hypertrophied compared to the control ones by 2.4 times ($p <0.05$). **Conclusions:** Alloxan-induced hyperglycemia leads to the development of emphysematous changes in combination with restriction. The vascular wall of muscular pulmonary vessels was thickened due to hypertrophy of the tunica media and tunica externa. The hypertrophy of lymphoid follicles and lymph nodes of the upper and lower mediastinum was observed in experimental animals.

Keywords: Lung, alloxan, hyperglycemia, age.

Introduction

Diabetes mellitus is a global medical and social problem because it leads to disability and death. It is ranked third after cardiovascular diseases and cancer. Approximately 300 million individuals are suffering from diabetes mellitus worldwide [1]. Type 1 Diabetes Mellitus mainly affects children and adolescents, reaching 1 million patients worldly [2, 3]. Diabetes mellitus is the basis for the development of complex comorbidities and complications such as diabetic microangiopathy and neuropathy. Diabetes patients are at increased

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risk for atherosclerosis, coronary heart disease, and renal failure [4-10]. For about half a century, scientists around the world have been thoroughly engaged in the problem of hyperglycemia, which is the consequence of diabetes mellitus type 1, but the causes of its occurrence have not been fully established yet. The most relevant modern theory is the autoimmune theory of diabetes pathogenesis [11, 12]. The interest in solving the problem of type 1 diabetes mellitus is not diminishing and encourages scientists to search for new ways of diagnosis, treatment and prevention. Violations of all kinds of metabolism in chronic hyperglycemia lead to generalized lesions of the macro- and microcirculation [13, 14]. Diabetic angiopathies have been thoroughly studied in the kidneys, retina, skin, and spleen, while changes in the blood vessels of the pulmonary circulation and, consequently, in the lungs are less explored.

Therefore, our main aim was to investigate the lungs of pre-reproductive age rats under the influence of alloxan-induced hyperglycemia.

Material and Methods

Twelve rats at the age of 3 months were divided into two series: experimental and control. Six experimental rats were served as an animal model of alloxan-induced hyperglycemia. The Control series includes six intact rats. For the experimental modeling of hyperglycemia, which was due to absolute insulin deficiency in the body, we used alloxan monohydrate. Aloxane monohydrate is a decomposition product of uric acid, which, through a parenteral route of administration, causes selective necrosis of pancreatic islets by interacting with the thiol glucokinase groups of the β-cell enzyme. As a result, glucose oxidation, ATP generation, and insulin secretion are reduced. Alloxan-induced hyperglycemia was simulated by injection of alloxan, pre-dissolved in 0.9% solution of sodium chloride, intraperitoneally once at a dose of 20 mg/100 g on an empty stomach. It is known that alloxan is toxic to cells of the tubules of the nephron [15]. Therefore, the animals additionally received a 10% glucose solution during the 24 hours after alloxan injection and 5% glucose solution during the experiment, which lasted 60 days. We measured the glucose level using Accu-Chek Advantage (Boehringer, Germany) at 2, 12, and 24 hours after alloxan injection, and then weekly. The average level of glucose in the blood remained at 11 mmol / L ± 2 mmol/L.

To study the remodeling of the lungs under hyperglycemia, we used the following methods:

- Histological examination. Histological samples prepared by the standard method were stained with hematoxylin and eosin, investigated using the light microscope (Olympus BH-2), and photographed with a digital video camera (Baumer Optronic CX 05c, Switzerland).
- Histomorphometry. It was performed dynamically using the universal certified program "SEO Scan Lab 2.0" and "SEO Image Lab 2.0". We measured the width and depth of pulmonary alveoli, the width of the conducting part of the respiratory bronchioles, the thickness of the interalveolar septum, perimeter of the lymphoid follicles, the thickness of the complex tunica intima + tunica media of the muscular pulmonary arteries, the thickness of the tunica media of vessels, and the thickness of the tunica externa of vessels.
- A statistical method. The obtained data were processed on a personal computer using the software "GraphPad" and a licensed Excel XP spreadsheet editor (Microsoft Office 2013). The data were analyzed by unpaired t-test. P values ≤ 0.05 were considered statistically significant.

Ethical approval

This study was approved by the Ethics Committee of the Sumy State University, Ukraine. The keeping and all manipulations of animals were carried out in accordance with generally accepted recommendations, requirements and provisions set out in the following documents: "European Convention of the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1985) and "General Ethical Principles of Animal Experiments" adopted by the First National Congress of Bioethics (Kiev, 2001).

Results

The light microscopy of lung tissue samples reveals full-blooding, thickening of the interalveolar septa with single inclusions of collagen fibers in their composition comparing with samples of intact animals. The lumen of the alveoli is free (Figures 1, 2). Along with emphysematous changes, we found collapsing alveoli. The interalveolar septa are of different thicknesses: thin in the areas of the expanded alveoli, and thick and full-blooded in atelectatic areas. Stasis in the vessels can be observed as well. Different-sized clusters of

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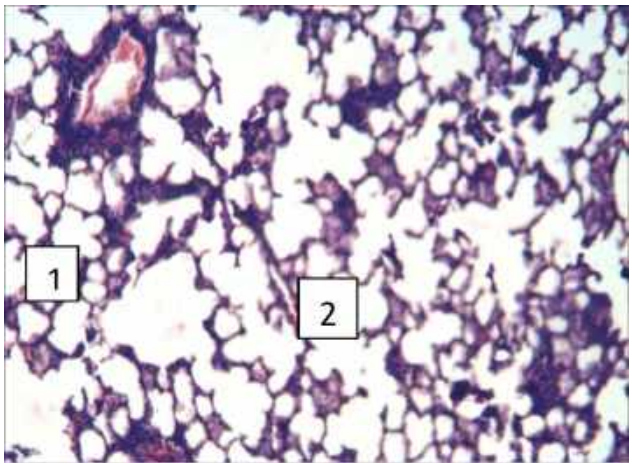

Figure 1: Lung's tissue of experimental rats ×100. 1 - Pulmonary alveoli cavity; 2 - interalveolar septum.

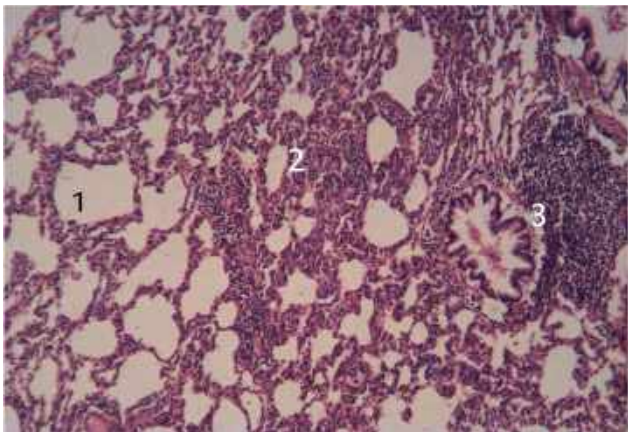

Figure 3: Lung's tissue of experimental rats ×100. 1 - Emphysematous transformation of lung tissue; 2 - areas of atelectasis; 3 - hypertrophied lymphoid follicle.

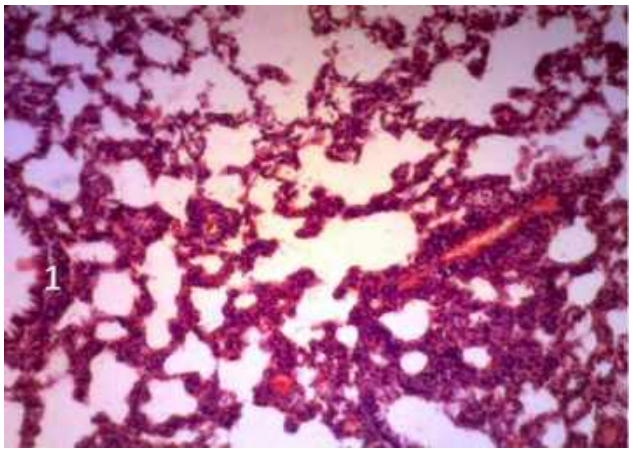

Figure 2: Lung's tissue of experimental rats ×100. 1 - Thickened and full-blooded interalveolar septa.

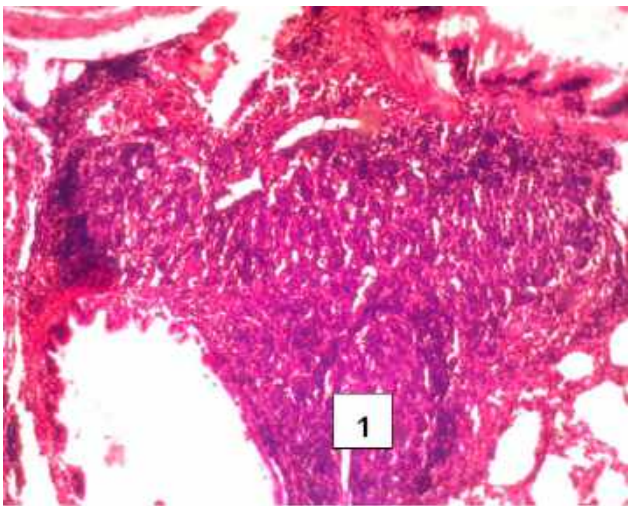

Figure 4: Lung's tissue of experimental rats ×100. 1 - Hypertrophied, full-blooded pulmonary lymphoid follicle surrounding the bronchus in the form of a "clutch".

lymphoid tissue are found around bronchi and vessels. They contain a large number of vessels (Figures 3, 4).

Inside the interalveolar septa, there is a small number of mature collagen fibers. In the tunica externa and tunica media of muscular pulmonary vessels is the hypertrophy of mature collagen fibers (Figure 5).

Obtained morphometric data show the increase of the width of alveoli by 8.5% ($p < 0.05$), the width of the conducting part of the respiratory bronchioles by 15.7 % ($p < 0.05$) and the thickness of the interalveolar septum by 8.3% ($p < 0.05$). Their values were 72.1 ± 0.46 µm, 62.003 ± 0.16 µm, and 6.9 ± 0.04 µm, respectively. This indicates the initial emphysematous changes in combination with fibrotic changes in the pulmonary stroma. The wall thickness of pulmonary vessels increased by 22.9% ($p < 0.05$) compared to intact animals and is 41.8 ± 0.02 µm ($p < 0.05$); accordingly, the thickness of its components increases. Thus, the thickness of the com-

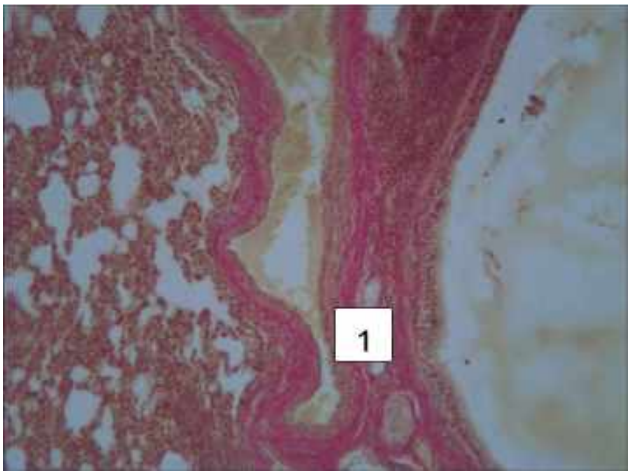

Figure 5: Lung's tissue of experimental rats ×100. 1 – Collagen fibers in the wall of the pulmonary vessel.

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plex tunica intima + tunica media of the muscular pulmonary vessels was 24.4 ± 0.01 μm ($p < 0.05$), which is 28.9% ($p < 0.05$) more than in intact rats. The increase in this indicator is mainly due to the thickening of the muscular layer, which is higher than the control indicator by 22.3% ($p < 0.05$) and is equal to 22.3 ± 0.02 μm ($p < 0.05$). The thickness of the tunica externa of vessels was 17.4 ± 0.02 μm ($p < 0.05$). The morphometry of lymphoid nodules indicates their hypertrophy, 1049.6 ± 0.16 μm ($p < 0.05$), which is 2.4 times ($p < 0.05$) more compared to the intact group.

Discussion

In the present study, we used such age groups as premature (3-month) animals. This choice is based on the fact that alloxan causes type 1 diabetes mellitus, which mainly affects this group of the population, while in older adults, type 2 diabetes mellitus is dominant [16].

The current study describes the processes of lung tissue remodeling, which are insufficiently covered in the scientific literature. Changes in the lungs occur primarily due to microcirculatory disorders in the pulmonary circulation under the influence of persistent hyperglycemia and are associated with the pro-inflammatory and proliferative properties of diabetes [17, 18]. The light microscopy with morphometry reveals sclerotic changes in the pulmonary stroma, destruction in the alveolates, which leads to the thickening of the interalveolar septa. Alloxan-induced hyperglycemia leads to the development of emphysematous changes in combination with restriction. The vascular wall of the muscular pulmonary vessels is thickened due to hypertrophy of the tunica media and tunica externa. The hypertrophy of lymphoid follicles and lymph nodes of the upper and lower mediastinum was observed in experimental animals. This is because the products of impaired glucose metabolism (advanced glycation end products - AGEs) are formed on the background of hyperglycemia. They bind to macrophage receptors, which in turn, secrete cytokines that provoke the development of immune responses [19-21].

Given the described changes, we can assume that microcirculatory disorders represent the trigger mechanism of lung tissue remodeling. Alloxan-induced hyperglycemia adversely affects the morphofunctional state of the lungs, both directly and indirectly (due to disorders of the cardiovascular system). Although the symptoms of complications from prolonged exposure to hyperglycemia are more pronounced in internal organs like the kidneys, brain, heart, we need to take into account the achieved findings since the effects of hyperglycemia on lungs lead to morphological remodeling and then, with the progression of diabetes, to the development of respiratory failure. This fact is confirmed in modern scientific works [22, 23].

Conclusion

The obtained results suggest that alloxan-induced hyperglycemia affects the structure of the lung tissue. Changes in the parenchymatous component of the lung tissue are mosaic. They are characterized by the development of emphysematous changes and the presence of sites of atelectasis. Muscle-type vessels and lymphoid tissue undergo hypertrophic changes.

Conflict of Interest

The author declares no conflict of interest.

References

1. Guimaraes JFC, Muzio BP, Rosa CM et al. Rutin administration attenuates myocardial dysfunction in diabetic rats. Cardiovascular Diabetology 14: 1–6, 2015
2. IDF Diabetes Atlas, Eighth edition, 2017. Online version of IDF Diabetes Atlas: www.diabetesatlas.org
3. Smiyan OI, Loboda AM, Manko YA et al. Dynamics of content of some minerals in teenagers with cardiovascular system pathology against the background of chronic tonsillitis. Wiadomosci lekarskie 71(4): 861-864, 2018
4. Papadopoulou-Marketou N, Margeli A, Papassotiriou I, Chrousos GP, Kakana-Gantenbein C, Wahlberg J. NGAL as an Early Predictive Marker of Diabetic Nephropathy in Children and Young Adults with Type 1 Diabetes Mellitus. J Diab Res 2: 11–17, 2017
5. Sattar N. Revisiting the links between glycaemia, diabetes and cardiovascular disease. Diabetologia 56: 686-695, 2013
6. de Ferranti SD, de Boer IH, Fonseca V et al. Type 1 Diabetes Mellitus and Cardiovascular Disease: A Scientific Statement From the American Heart Association and American Diabetes. Association Diabetes Care 37(10): 2843-2863, 2014
7. Guzyk MM, Dyakun KO, Yanytska LV et al. Inhibitors of Poly(ADP-Ribose)Polymerase-1 as Agents Providing Correction of Brain Dysfunctions Induced by Experimental Diabetes. Neurophysiology 49(3): 183-193, 2017
8. Cherkashyna L, Konoval N, Shklyar A et al. Study of structural and biochemical changes in the muscular tissue of the oesoph-

 https://doi.org/10.46389/rjd-2020-1008 © 2020 The Authors

agus for solving the problem of diagnosing the prescription of death coming Pol Merkur Lekarski 48 (283): 42-44, 2020
9. Yarmolenko O, Sikora V, Bumeister V, Olga P. Age-dependent cardioprotective action of meldonium on heart remodeling under the experimental hypoosmolar hyperhydration. Bangladesh J Med Sci 18(2): 395-401, 2019
10. Barchan G, Demikhov O, Cherkashyna L et al. A complex of regional ecological and medico-social factors: evaluation of dysplastic dependent pathology of the bronchopulmonary system Pol Merkur Lekarski 48 (283): 49-54, 2020
11. Okey AO, Paul CC, Agomuo CO. Blood glucose level and lipid profile of alloxan-induced hyperglycemic rats treated with single and combinatorial herbal formulations Journal of Traditional and Complementary Medicine 6(2): 184-192, 2016
12. Demikhova N, Cherkashyna L, Chernatska O et al. The relationship between lipid metabolism and albuminuration level with single nucleotide polymorphism -204a>c [rs 3808607] CYP7A1 gene in patients with 2 type diabetes mellitus and diabetic nephropathy Rom J Diabetes Nutr Metab Dis 26(3): 253-261, 2019
13. Chernatska O, Demikhova N Improvement of treatment in persons with arterial hypertension and type 2 diabetes mellitus. Georgian Medical News 11(284): 47-51, 2018
14. Chernatska O, Demikhova N, Rudenko T, Demikhov A Assesment of the lipid profile correction in patients with arterial hypertension and type 2 diabetes mellitus Azerbaijan Medical Journal 1: 95-99, 2019
15. Misra M, Aiman U. Alloxan: An unpredictable drug for diabetes induction? Indian Jornal of Pharmacology 44(4): 538-539, 2012
16. Wenjun F. Epidemiology in diabetes mellitus and cardiovascular disease. Cardiovascular Endocrinology & Metabolism 6 (1): 8-16, 2017
17. Khateeb J, Fuchs E, Khamaisi M. Diabetes and Lung Disease: A Neglected Relationship. Rev Diabet Stud. 15:1-15, 2019
18. Marushchak M, Krynytska I, Petrenko N, Klishch I. The determination of correlation linkages between level of reactive oxygen species, contents of neutrophils and blood gas composition in experimental acute lung injury Georgian Medical News 4(253): 98-103, 2016
19. Kohlmeier JE, Miller SC, Woodland DL. Cutting edge: antigen is not required for the activation and maintenance of virus-specific memory CD-8 T-cells in the lung airways. J Immunol 178(8): 4721-5, 2007
20. Krynytska I, Marushchak M. The Indices of Nitric Oxide System in Rats with Carrageenan-Induced Enterocolitis Combined with Diabetes Mellitus. Rom J Diabetes Nutr Metab Dis 25(3): 283-288, 2018
21. Prykhodko OA, Gulaya VI, Yarmolenko OS et al. Microscopic changes in the organs of rats under conditions of general dehydration of the organism Azerbaijan Medical Journal 4: 95-100, 2016
22. Te-Wei H, Chun-Ta H, Sheng-Yuan R, Yu-Ju T, Feipei L, Chong-Jen Y. Diabetes mellitus in patients with chronic obstructive pulmonary disease-The impact on mortality. PLoS ONE 12(4), 2017
23. Kinney GL, Black-Shinn JL, Wan ES et al. Pulmonary Function Reduction in Diabetes With and Without Chronic Obstructive Pulmonary Disease Diabetes Care 37(2): 389-395, 2014

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Original Article

The Relationship between Non-Linear Analysis of Heart Rate Variability, QTc Interval and Cardiovascular Risk Factors in Young Individuals with Pre-Diabetes

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Abstract

Introduction: Cardiac autonomic functions and cardiovascular risk factors are closely associated with each other. This study aimed to evaluate the cardiac autonomic status employing the Poincaré plot and QTc in young pre-diabetic individuals and correlate it with the cardiovascular risk factors. **Material and Methods:** This was a cross-sectional study. The students participating in the health check-up program organized by the college were the study participants. Basal anthropometric measurements, detailed family, and personal medical history were documented. Autonomic functions were evaluated. Plasma glucose and lipid profile were evaluated biochemically. Based on the impaired fasting plasma glucose and impaired glucose tolerance values, subjects were classified as normal and pre-diabetes mellitus groups. **Results:** A total of 295 subjects (198 normal and 97 pre-diabetes mellitus), were evaluated. Standard descriptor 1 and 2 in the pre-diabetes mellitus group reported a significant decrease, p<0.0001, (95% CI 15.98, 19.07) (95%CI 31.73 37.26) compared to the normal group (95% CI 26.33, 30.27) (95% CI 48.39, 52.71). QTc was significantly increased in the pre-diabetes mellitus group, p<0.0001, (95% CI 415.62, 423.99). Body mass index, fasting plasma glucose, and lipid parameters reported as being significant independent variables were associated with autonomic function test parameters. **Conclusion:** Cardiac autonomic dysfunction starts appearing in the pre-diabetic stage itself. Body mass index and altered lipid profiles showed a significant association with increased blood glucose levels. Early detection at a young age can help to plan better prevention and treatment strategies.

Keywords: Impaired fasting glucose, Oral glucose tolerance, QTc interval, Heart rate variability.

Introduction

In India, 69.1 million individuals have diabetes, making it the Diabetic Capital of the world [1]. The reasons for the alarming increase may be due to rapid socio-economic and nutritional transition, lack of self-awareness, and follow-up programs in the society. As per the "ticking clock hypothesis", the microvascular disease manifestation appears in the precursor stage, before the expression of full-blown clinical type 2 diabetes mellitus [2]. According to the American Diabetes Association, pre-diabetes is Impaired Fasting Glucose (IFG) ranging from 100–125 mg/dL or Impaired Glucose Tolerance (IGT) of 140– 199 mg/dL [3]. Pre-diabetes is related to increased cardiovascular (CV) disease and mortality [4]. Evaluation strategies targeting the younger candidates with increased risk will be a potential boon for the society to plan early interventional strategies.

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The autonomic nervous system, with its sympathetic and parasympathetic limbs, orchestrates numerous physiological and pathological cardiovascular responses. The sympathovagal imbalance is an indicator of increased cardiovascular risk. A variety of markers have been provided to reflect autonomic activity and cardiovascular risk, that includes heart rate, heart rate variability (HRV), [5] QT interval lengthening, and QTc (Corrected QT interval) [6].

Early diagnoses of autonomic dysfunction employing the Ewing test battery are outdated. HRV has been widely accepted as a non-invasive marker of autonomic control [5]. HRV represents the variation in time between successive heartbeats. The traditionally employed linear analysis method (time and frequency domain) is prone to interference by ectopic rhythms and assumes that the analyzed R-R segments are stationary, and variations occur in a harmonic fashion. Considering the fact that the cardiovascular system exhibits non-linear dynamics, the newly explored non-linear methods of HRV analysis provide better insights into the autonomic control of the cardiovascular system. Poincare plot is a geometric, semi-quantitative method, used for identifying the non-linear patterns within the electrocardiograph (ECG) data [7].

QT interval is an ECG measurement that represents the duration between ventricular depolarization to ventricular repolarization. It is affected by heart rate, so QTc calculated using Bazett's square root formula is the accepted gold standard that reflects autonomic activity.

With this perspective, the current study was aimed to evaluate the cardiac autonomic status employing Poincare plot and QTc in young pre-diabetic individuals. Also, we sought to identify the cardiovascular risk factors that contribute to autonomic dysfunction.

Material and Methods

Study design and patients

This was a cross-sectional study. Bachelor of Medicine, Bachelor of Surgery students participating in the health check-up program organized by the institution, were the study participants. The study was approved by the Institute Ethics Committee of the Sri Manakula Vinayagar Medical College and Hospital.

A total of 403 participants from the 18-25 age group were screened during the medical health check-up program. Sociodemographic details, personal history, general examination, systemic examination, family history of diabetes, and blood investigations (fasting blood glucose and lipid profile) were evaluated in all individuals on the day of the health check-up program. Seventy-six participants were excluded due to a history of any medication intake, any form of medical illness, athletes, fasting glucose more than 125mg/dL, or if they reported having cardiovascular diseases. Among the remaining 327 subjects, 32 participants were not willing to participate after hearing the protocol procedures. The remaining 295 subjects were the study participants. Based on their fasting pasma glucose (FPG) and IGT values, the subjects were divided into two groups; Group I (n=198) included subjects who had FPG and IGT in the range of 60-99 mg/dL and >140 mg/dL respectively; Group II (n=97) included subjects with FPG and IGT ranging from 100-125 mg/dL or 140–199 mg/dL, respectively.

Cardiovascular autonomic function test: HRV Analysis - Poincare plot

The study was carried in the Autonomic Laboratory, Department of Physiology, Sri Manakula Vinayagar Medical College and Hospital, between 07.00 to 8:30 A.M. The Task Force guidelines on HRV were followed. The subject was given rest for 10 minutes, and lead II ECG was obtained using Recorders and Medicare (RMS) polyrite D hardware, version 1.0, India. The sampling rate of 500Hz and bandpass filter of 2Hz to 40Hz was followed. The readings were taken thrice each with a five minutes interval, and the last 5 minutes ECG was performed, digitalized, and stored for HRV analysis. From the ECG reading, the RR series were extracted with maximum amplitude and sharpness. Analysis of the RR series was done using the Finland version 1.1 software for HRV (Bio-Signal Analysis Group, University of Kuopio, Finland).

The Poincaré plot represents a time series on a cartesian plane. It is a scatter plot of RRn vs. RRn+1. The time between two successive R peaks is represented by RRn, and the time between the next two successive R peaks is represented by RRn+1.Employing the ellipse-fitting technique on the plot, two main indices are depicted: the standard deviation of instantaneous beat-to-beat interval variability (SD1), and the continuous long-term R/R interval variability (SD2). On the Poincaré plot, SD1 is the width and SD2 the length of the ellipse. The line of identity (LOI) is the 45° imaginary diagonal line on the Poincaré plot. SD1 depicts short-term HRV and measures the dispersion of points

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along the line perpendicular to the LOI and is considered as an indicator of parasympathetic activity, whereas SD2 portrays both long and short-term HRV and overall variability and assesses dispersion of points along with the LOI [8].

ECG Analysis

A 12 lead ECG recording was obtained using automated ECG, RMS Vesta 302i. ECG paper speed was kept at 25 mm/sec. The QT interval represents the duration from the beginning of the QRS complex to the end of the T wave. QTc was measured using the Bazett formula: QT / √RR.

Body composition analysis

Body composition was analyzed using the HBF-375, Karada Scan Body Composition Monitor. The subjects were asked to stand barefooted on the foot electrodes and hands holding the grip electrodes in such a way that the arms and the body are kept at 90°. Body mass index (BMI), body fat, and visceral fat were evaluated automatically based on bioelectric impedance.

Biochemical Investigations

Five ml of venous blood were withdrawn, and biochemical investigations were carried out.

Serum glucose

The subjects were instructed for 8 hours of overnight fasting, and a blood sample was collected for estimating fasting blood glucose (FBG) levels. The subjects were then administered 75 g of anhydrous glucose in 250 to 300 mL, and a blood sample was collected again 2 hours after the test load. The FBG and oral glucose tolerance test values were measured using the glucose oxidase-peroxidase method using reagent kits supplied by Accurex Biomedical Pvt Limited, Mumbai, adapted to the Cobas Mira Plus Automated Chemistry analyzer.

Lipid profile

Serum total cholesterol (TC) was evaluated by the cholesterol oxidase method, and triglyceride (TG) levels were measured by the glycerol kinase- peroxidase method. High-density lipoprotein cholesterol (HDL-c) was measured by the divalent cation precipitation method using reagent kits adapted to an automated blood analyzer. Very low-density lipoprotein cholesterol (VLDL-c) was calculated by dividing the triacylglycerol concentration by 5 and low-density lipoprotein cholesterol (LDL-c) by using Friedwald's equation [TC-(VLDL+HDL)].

Statistical analysis

Continuous data were expressed as mean (Standard Deviation) and categorical data as frequencies. Frequency distributions between the groups were compared using the $\chi 2$ test.

The comparison of basic physiological parameters, cardiovascular autonomic function test parameters, and biochemical parameters for normally distributed data was made using the student t-test. The association was evaluated using Pearson correlation analysis. Univariate regression analysis was used to evaluate the contribution of various CV risk factors on the autonomic function.

Statistical package for social sciences (SPSS) version 19.0 for Windows (SPSS Inc., USA) was used for data analysis. A p-value of less than 0.05 was considered statistically significant.

Results

Out of the 295 individuals, 198 were normal, and 97 were pre-diabetic. The pre-diabetic subjects had significantly higher BMI; [95% CI (22.01, 22.95) vs. (28.45, 30.18) (p=<0.0001)], when compared to the normal group (Table 1).

The SD1 and SD2 was found to be significantly decreased among the pre-diabetic group: [95%CI (26.33, 30.27) vs. (15.98, 19.07)], [95%CI (48.39, 52.71) vs. (31.73, 37.26)], [95%CI (20.05, 20.95) vs. (14.03, 15.16)], respectively compared to the normal group. QTc was significantly prolonged among the pre-diabetic group compared to the normal. [95%CI (383.93, 391.20) vs. (415.62, 423.99)] (Table 2).

TC, TG, LDLc, VLDLc, BF, and VF reported a significant increase among the pre-diabetic group, while HDLc showed a decrease (Table 3). A significant correlation was reported between autonomic function, age, FPG, and BMI. SD1 and SD2 were negatively correlated to age, FPG, and BMI. QTc showed positive correlation with age (r=0.24), FPG (r=0.49) and BMI (r=0.37) (Table 4).

Univariate regression analysis showed that age, FPG, BMI, and lipid profile parameters were significantly associated with SD1, SD2, and QTc (Tables 5 and 6).

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Table 1: Basal anthropometric measurements and BP among the two groups.

Parameters	Control (n=198)	preDM (n=97)	p-value
	Mean (SD)	Mean (SD)	
Age, yr	19.19 (1.19)	20.33 (1.32)	<0.0001
FPG, mg/dL	80.94 (3.29)	110.66 (5.75)	<0.0001
IFG, mg/dL	97.21 (21.6)	150.44 (13.12)	<0.0001
SBP, mmHg	109.51 (6.51)	119.39 (9.55)	<0.0001
DBP, mmHg	66.84 (6.57)	71.45 (8.17)	<0.0001
HR, beats/min	76.01 (6.67)	77.81 (8.16)	0.04
WC, cms	82.52 (9.66)	86.12 (22.88)	0.05

Note: Values are expressed as mean (SD). P value less than 0.05 was considered to be significant. PreDM, Prediabetic; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; WC, waist circumference; HC, hip circumference; BMI, body mass index.

Table 2: Autonomic function evaluated using the Poincaré plot and QTc among the two groups.

Parameters	Control n=198		PreDM n=97		p-value
	Mean(SD)	95%CI	Mean(SD)	95%CI	
SD1 (ms)	28.30 (14.04)	26.33, 30.27	17.53 (7.66)	15.98, 19.07	<0.0001
SD2 (ms)	50.55 (15.38)	48.39, 52.71	34.49 (13.71)	31.73, 37.26	<0.0001
QRS (ms)	97.67 (17.16)	95.26, 100.07	102.76 (23.37)	98.05, 107.47	0.03
QT (ms)	345.58 (32.65)	341.01, 350.16	364.41 (39.91)	356.10, 372.19	<0.0001
QTc (ms)	387.56 (25.93)	383.93, 391.20	419.80 (20.78)	415.62, 423.99	<0.0001

Note: Values are expressed as mean±SD. P-value less than 0.05 was considered to be significant. SD1, standard descriptor 1; SD2, standard descriptor 2; QTc, corrected QT interval.

Table 3: Lipid profile, body fat and visceral fat among the two groups.

Parameters	Control n=198		preDM n=97		p-value
	Mean (SD)	95% CI	Mean (SD)	95%CI	
TC	133.76 (16.38)	131.47,136.06	172.94 (12.18)	169.92, 176.60	<0.0001
TG	77.42 (24.85)	73.94, 80.91	103.68 (39.25)	95.77, 111.59	<0.0001
HDLc	37.56 (3.47)	36.85, 38.25	35.65 (4.52)	35.01, 36.28	<0.0001
LDLc	77.49 (9.91)	76.53, 79.01	84.95 (9.96)	82.94, 86.95	<0.0001
VLDLc	15.48 (4.97)	14.78, 16.18	20.73 (7.85)	19.15, 22.31	<0.0001
BodyFat (%)	28.66 (7.02)	27.67, 29.64	31.38 (7.13)	29.74, 32.50	0.002
Visceral Fat	4.35 (2.84)	3.95, 4.74	10.71 (4.91)	9.73, 11.71	<0.0001

Note: P value less than 0.05 was considered to be significant. TC, total cholesterol; TG, trilycerides; HDLc, high density lipid cholesterol; LDLc, low density lipid cholesterol; VLDLc, very low density lipid cholesterol.

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Table 4: Pearson's correlation between autonomic function, age, FPG, IGT, BMI and HR.

	SD1		SD2		QTc	
	r	p	r	p	r	p
Age	-0.21	0.0001	-0.22	0.0001	0.24	0.0001
FPG	-0.42	0.0001	-0.46	0.0001	0.49	0.0001
IGT	-0.14	NS	-0.17	NS	0.12	NS
BMI	-0.42	0.0001	-0.47	0.0001	0.37	0.0001
Heart rate	-0.12	0.03	-0.09	0.11	0.15	0.08

Note: P-value less than 0.05 was considered to be significant.

Table 5: Univariate regression analysis with SD1 and SD2 as dependent variables and age, FPG, BMI and lipid profile as independent variables.

Independent variables	Standardized Beta coefficients	95% CI		p-value
		Lower bound	Upper bound	
SD1 as Dependent Variable				
Age	-0.21	-3.21	-0.98	0.0001
FPG	-0.41	-0.47	-0.28	0.0001
BMI	-0.42	-1.34	-0.81	0.0001
TC	-0.23	-0.18	-0.06	0.0001
TG	-0.12	-0.09	-0.01	0.04
HDLc	0.08	-0.11	0.61	0.17
LDLc	-0.31	-0.56	-0.27	0.0001
SD2 as Dependent Variable				
Age	-0.21	-3.95	-1.16	0.0001
FPG	-0.46	-0.64	-0.41	0.0001
BMI	-0.47	-1.84	-1.19	0.0001
TC	-0.35	-0.30	-0.16	0.0001
TG	-0.19	-0.16	-0.04	0.0001
HDLc	-0.01	-0.48	0.41	0.88
LDLc	-0.29	-0.69	-0.31	0.0001

Note: P-value less than 0.05 was considered to be significant.

Table 6: Univariate regression analysis with QTc as dependent variable and age, FPG, BMI and lipid profile as independent variables.

Independent variables	Standardized Beta coefficients	95% CI		p-value
		Lower bound	Upper bound	
QTc as Dependent Variable				
Age	0.24	2.78	7.53	0.0001

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FPG	0.49	0.77	1.16	0.0001
BMI	0.36	1.42	2.61	0.0001
TC	0.45	3.39	0.63	0.0001
TG	0.32	0.19	0.38	0.0001
HDLc	0.11	-0.02	1.51	0.05
LDLc	0.16	0.13	0.79	0.01

Note: P-value less than 0.05 was considered to be significant.

Figure 1 represents the Poincaré plot among the normal and preDM groups, with its numerical descriptors SD1 and SD2.

Discussion

In the present study, the prevalence of prediabetes was observed to be 32.88%, with 66% of males, indicating an alarming increase in the pre-diabetic population. A previous study from India, comparing the risk factors in the Indian and U.S. population, reported a prevalence of prediabetes of 24% [9]. The CV risk factors showed significant changes among the pre-diabetic group compared to the normal group, further supporting the "ticking clock hypothesis" [2].

In our study, BMI, which represents general obesity, and visceral fat and waist circumference (WC), that represents central obesity, were significantly increased in the preDM group. This is in agreement with other studies from the literature, which report that BMI is a significant risk factor for diabetes [10] and is also correlated to preDM [11]. It was found that BMI was significantly positively correlated to QTc and negatively correlated to the SD1 and SD2 parameters. Similar findings were reported by Ravikumar et al., where BMI was positively correlated to QTc [12].

We found significant differences in SD1 and SD2 among the two groups. The lower SD1 in the preDM group indicates weakened parasympathetic regulation, and lower SD2 indicates increased sympathetic activity depicting that there is a pronounced vagal withdrawal and sympathetic augmentation in the pre-diabetic stage. Also, in our study, SD1 and SD2 reported a significant moderate correlation with BMI and FPG. Univariate regression analysis revealed that FPG causes a 17% variation in SD1 and a 21% variation in SD2. A study among diabetic patients employing the non-linear analysis of HRV suggested decreased parasympathetic modulation and suggested using non-linear methods to evaluate autonomic dysfunction in diabetic individuals [13]. Autonomic impairment is reported even before the alteration in glucose homeostasis becomes clinically apparent [14]. A study conducted on 400 subjects to find the role of isolated IFG on the pathogenesis of cardiac autonomic neuropathy (CAN), investigating HRV and heart rate turbulence observed that autonomic dysfunction is present in the isolated IFG subtype of preDM [15]. A recent update on diabetes has put forward the putative mechanisms for CAN, which include tachycardia, QT interval, impaired HRV, reverse dipping, and orthostatic hypotension [16]. A review suggests that since CAN is a predictor of adverse cardiac functioning, an improvement in ANS can thus help to reduce CV events in the future [17].

QTc showed a significant increase among the preDM group compared to the normal group, but the increase was within the normal limits, not clinically significant. The QTc interval showed a significant positive correlation with FPG and a moderate correlation with BMI. QTc prolongation may be due to the sympathetic dominance among the preDM group. It is observed that prolonged QTc duration and QTc dispersion is related to increased glycemic variability [18]. Increased long-term variability of postprandial glucose is an independent risk factor for QTc prolongation in type 2 diabetes [19]. A study on the Chinese population in 2012 showed that postprandial glucose level was an independent risk factor for prolongation of QTc interval, and the underlying mechanism may be due to increased reactive oxygen species, increased calcium concentration, and sympathovagal imbalance [20]. The exact basis of QTc prolongation in the preDM group and the effect of hyperglycemia is multifactorial.

This study reported cardiac autonomic dysfunction in younger individuals employing the Poincaré plot analysis and QTc. The limitations include the small sample size and the fact that gender-based analysis of autonomic function was not carried out; also, dietary practices and physical activity were not taken

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into account, and since it is a cross-sectional study, the exact cause and pathophysiological link behind IFG and autonomic functions could not be elucidated. Future longitudinal studies are required to explain the link between hyperglycemia and impaired autonomic function.

Conclusion

Autonomic dysfunction appears in the pre-diabetic stage, in the form of parasympathetic withdrawal and sympathetic dominance. Gender, BMI, and FPG were reported to show a significant association with SD1, SD2, and QTc. This study emphasizes glucose monitoring and evaluating autonomic functions, in younger individuals to know the extent of alterations caused and to plan early interventional strategies.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. International Diabetes Federation. ID Diabetic Atlas 7th Edition. http://www.idf.org/idf-diabetes-atlas-seventh-edition.
2. Sorensen BM, Houben AJ, Berendschot TT et al. Prediabetes and type 2 diabetes are associated with generalized microvascular dysfunction: the Maastricht study. Circulation 134(18I): 1339–1352, 2016.
3. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 34 [Suppl 1]: S62–69, 2011.
4. Huang Y, Cai X, Mai W, Li M, Hu Y. Association between prediabetes and risk of cardiovascular disease and all cause mortality: systematic review and meta-analysis. BMJ 355: i5953, 2016.
5. Trivedi GY, Saboo B, Singh RB, Maheshwari A, Sharma K, Verma N. Can decreased herat rate variability be a marker of autonomic dysfunction, metabolic syndrome and diabetes? J Diabetol 10(2): 48-56, 2019.
6. Karita K, Iwata T, Maeda E, Sakamoto M, Murata K. Assessment of Cardiac Autonomic Function in Relation to Methylmercury Neurotoxicity. Toxics: 6(3): 38, 2018.
7. Roy B, Ghatak S. Nonlinear methods to assess changes in heart rate variability in type 2 diabetic patients. Arq Bras Cardiol 101(4): 317-327, 2013.
8. Lo PC, Tsai PH, Kang HJ, Miao Tan WJ. Cardiorespiratory and autonomic-nervous-system functioning of drug abusers treated by Zen meditation. J Tradit Complement Med 9(3): 215–220, 2018.
9. Gujral UP, Narayan KMV, Pradeepa RG, et al. Comparing Type 2 Diabetes, Prediabetes, and Their Associated Risk Factors in Asian Indians in India and in the U.S.: The CARRS and MASALA Studies. Diabetes Care 38(7): 1312-1318, 2015.
10. Little M, Humphries S, Patel K, Dudd W, Dewey C. Factors associated with glucose tolerance, pre-diabetes, and type 2 diabetes in a rural community of south India: a cross-sectional study. Diabetol Metab Syndr 8: 2, 2016.
11. Sultana S, Kulkarni PK. Prevalence of Pre-diabetes (Impaired fasting glucose and/or Impaired glucose tolerance) among urban slum dwellers. Diabetes Cholest metabol 1: 103, 2016.
12. Ravikumar V, VajraVelu HR, Ayyavoo S, Ramraj B. Correlation of Adiposity Indices with Electrocardiographic Ventricular Variables and Vascular Stiffness in Young Adults. J Clin Diagn Res 11(6): CC21–CC24, 2017.
13. Bhaskar R, Ghatak S. Nonlinear Methods to Assess Changes in Heart Rate Variability in Type 2 Diabetic Patients. Arquivos Brasileiros de Cardiologia 101(4): 317-327, 2013.
14. Meyer ML, Gotman NM, Soliman EZ et al. Association of glucose homeostasis measures with heart rate variability among Hispanic/Latino adults without diabetes: the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Cardiovasc Diabetol 15: 45, 2016.
15. Balcıoğlu AS, Akıncı S, Çiçek D, Bal UA, Muderrisoglu IH. Cardiac autonomic nervous dysfunction detected by both heart rate variability and heart rate turbulence in prediabetic patients with isolated impaired fasting glucose. Anatol J Cardiol 16(10): 762–769, 2016.
16. Spallone V. Update on the Impact, Diagnosis and Management of Cardiovascular Autonomic Neuropathy in Diabetes: What Is Defined, What Is New, and What Is Unmet. Diabetes Metab J 43(1): 3–30, 2019.
17. Vinik AI, Casellini C, Parson HK, Colberg SR, Nevoret M-L. Cardiac Autonomic Neuropathy in Diabtes: A Predicdtor of Cardiometabolic events. Front Neurosci 12: 591, 2018.
18. Sertbas Y, Ozdemir A, Sertbas M, Dayan A, Sancak S, Uyan C. The Effect of Glucose Variability on QTc Duration and Dispersion in Patients with Type 2 Diabetes Mellitus. Pak J Med Sci 33(1): 22–26, 2017.
19. Su JB, Yang XH, Zhang XL, et al. The association of long-term glycaemic variability versus sustained chronic hyperglycaemia with heart rate-corrected QT interval in patients with type 2 diabetes. PLoS One 12(8): e0183055, 2017.
20. Li X, Ren H, Xu ZR, Liu YJ, Yang XP, Liu JQ. Prevalence and Risk Factors of Prolonged QTc Interval among Chinese Patients with Type 2 Diabetes. Exp Diabetes Res: 234084, 2012

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Original Article

Anthropometric Measurements and Body Composition of Indonesian Patients with Type 2 Diabetes are Associated with Inflammation, Independent of Sex and Disease Duration

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Abstract

Introduction: Abnormal body composition, such as a high percentage of body fat, triggers an inflammatory reaction, which links the association between obesity and diabetes complications. Females, in general, have higher body fat, which may play a part in elevating cardiovascular disease risk. This study aimed to study the association between several anthropometric measurements and inflammatory markers in individuals with type 2 diabetes mellitus. We intended to confirm if this association is independent of sex and duration of diabetes. **Material and Methods:** People with type 2 diabetes mellitus were recruited from primary health care centers in Sleman, Indonesia. Several anthropometric measurements were performed, followed by an analysis of plasma high-sensitivity C-reactive protein. A comparison of anthropometric measurements was made between individuals with (high-sensitivity CRP<3 mg/L) and without inflammation (high-sensitivity CRP≥3mg/L). Spearman correlation analysis was conducted to learn the association between anthropometric measurements with the high-sensitivity CRP level. Adjustments were made for sex and duration of diabetes. **Results:** Differences were found on weight, BMI, hip circumference, upper-arm circumference, and wrist circumference. Among body composition measurements, total body fat, subcutaneous fat (whole body, trunk and leg), and visceral fat were different between groups. Bodyweight, BMI, waist circumference, hip circumference, arm circumference, neck circumference, and wrist circumference were significantly correlated with high-sensitivity CRP values. **Conclusion:** Associations were found between the inflammatory marker and anthropometric measurements, independent of sex and disease duration.

Keywords: Diabetes, inflammation, C-reactive protein, anthropometry, body composition

Introduction

Inflammation is a natural mechanism against infection and injury. Inflammation is initiated and intensified in a short time following the insults, which can sustain for days. In a normal condition, the inflammatory response is swiftly terminated upon clearance of infection and injurious mediators. This careful control of complex inflammatory signaling cascades minimizes tissue damage as a consequence of free radicals released during inflammation [1].

A milder and sustained version of inflammation has been extensively studied within past decades, which is linked to several conditions, including cardiovascular diseases [2], cancer [3], Alzheimer's disease [4], sarcopenia [5] and type 2 diabetes mellitus [6]. This phenomenon is commonly termed chronic, low-grade inflammation, or metabolic inflammation (metaflammation). It drifts from the classic type of inflammation, as it lacks calor, dolor, rubor, and tumor. In addition to that, there is no substantial increase in basal energy expenditure observed [7]. A higher degree of chronic

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inflammation was observed with increasing age. This aging-associated chronic inflammation or inflammaging may result from genetic factors, central adiposity, leaky gut, gut dysbiosis, mitochondrial dysfunction, and chronic infection [8].

In type 2 diabetes mellitus (T2DM), inflammation may be triggered by several distinct mechanisms. Excessive adiposity increases the concentration of Interleukin-6 (IL-6) in the circulation [9, 10]. IL-6 induces an acute phase response, which is followed by the release of acute phase mediators such as serum amyloid A (SAA), haptoglobin, fibrinogen, and C-reactive protein (CRP) from the liver [11]. Poor glycemic control may lead to the formation of advanced glycation end products (AGEs), which can bind to the receptor of AGE (RAGE). RAGE ligation on vascular or immune cells triggers several signal transduction cascades leading to activation of NF-κB [12] and transcription of several inflammatory cytokines including IL-1, IL-2, IL-6, IL-8, IL-12 and TNF-α [13]. AGE formation is accelerated due to inflammation, oxidative stress, and aging [14].

There are differences between genders in terms of the risk and outcome of diabetes. These differences may be caused by organs' systemic response to a difference in sex chromosome and gender-specific expression of genes in the autosomes and sex-specific hormones [15]. These differences lead to phenotypic differences between genders, including body composition. Body fatness, which is commonly higher in females, is associated with diabetes outcomes. According to the UK Biobank Study, which investigated the differences of anthropometric adiposity measures (weight, waist and hip circumference, BMI, and body fat) between individuals with and without diabetes, the measure differences were higher in female participants [16]. Adiposity is positively correlated with chronic inflammation [17], which contributes to the sex-associated difference in inflammation state. Indeed, a previous study involving a large number of female and male participants with new-onset diabetes (13,676 and 7,809, respectively) showed that high-sensitivity C-reactive protein (hs-CRP) concentration tended to be higher in females (1.85 ±3.09 vs. 1.47±2.33; $p<0.000001$) [18]. In a systematic review of over five-million participants, women with diabetes have a 58% higher risk of developing coronary heart disease. Compared with their male counterparts, females with diabetes also have a 30% greater risk of cardiovascular disease. In addition to that, a 13% greater all-cause mortality risk is attributable to females with diabetes [19].

Increased risk of diabetes complications is also associated with a longer duration of diabetes. In a previous study, the duration of diabetes is associated with death, microvascular, and macrovascular events [20]. A Sri Lankan study showed that the duration of diabetes >10 years was associated with a doubled risk of microvascular complication and diabetic foot [21]. The risk of infectious diseases, such as infective endocarditis, is also elevated with longer duration of diabetes. A three-times higher risk of infective endocarditis is seen in individuals that have diabetes for more than 15 years compared with 0-5 years [22].

CRP, as a marker of inflammation, was shown to be associated with several diseases risk. CRP can be categorized into lower (<1 mg/L), moderate (1-3 mg/L), and higher (>3 mg/L), which represent a relative risk of heart disease [23]. In a meta-analysis using a fixed-effect model, the subjects in the highest category of hs-CRP had twice the risk of cardiovascular mortality. In the same study, cancer risk was increased by 25% for those in the highest category of hs-CRP [3].

In this study, we aimed to investigate the factors that may be related to inflammation in patients with type 2 diabetes mellitus, in this case, anthropometric measurements, muscle strength, and body composition. Associations were evaluated controlling for sex and duration of disease.

Material and Methods

Participants

Participants were recruited from two-state primary health care centers and one private primary care clinic in Sleman, Indonesia. At the time, they were participating in a Chronic Disease Management Program, an activity organized at primary health care level to promote health to people with chronic diseases including physical activity, health monitoring, medical consulting, psychological services, nutrition counseling, physiotherapy, and laboratory tests [24]. All male and female individuals diagnosed with type 2 diabetes were recruited to this study. Participation in this study was entirely voluntary. This study was approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (No. KE/FK/1253/EC/2018). All subjects gave written informed consent to participate in the study.

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Anthropometric Measurements

We measured participants' body weight, height, waist circumference, hip circumference, upper arm circumference, neck circumference, wrist circumference, handgrip strength, and triceps skinfold. All measurements were performed in compliance with the Body Measurements (Anthropometry) protocol published by the U.S. Center for Disease Control and Prevention [25]. Bioelectrical impedance analysis (BIA) was also performed to estimate whole body, trunk, arm and leg subcutaneous fat and muscle mass, visceral fat, and total body fat (Omron HBF 375, USA).

Laboratory method

Venipuncture by a phlebotomist was carried out for all subjects after 8 hours of fasting, using vacuum extraction tube systems. The blood in the tube containing EDTA was centrifuged at 2000 x g for 15 minutes, followed by the transfer of the supernatant into microtubes. The tubes were then frozen at 20°C until further analysis. Plasma high-sensitivity C-reactive protein analysis was performed using the Enzyme-linked immunosorbent assay (ELISA) method as instructed in the provided manual (Calbiotech, USA). The concentration of hs-CRP at or above 3 mg/L was considered inflammation.

Data analysis

Participants were divided into two groups according to inflammation status (hs-CRP <3 vs. ≥3 mg/L). Anthropometric measurement differences between groups were analyzed using the General Linear Model. Statistical significance was determined in three models: unadjusted, adjusted for sex, and adjusted for sex and duration of diabetes. Besides, partial Spearman's Correlation was used to analyze the association between anthropometric measurements with hs-CRP, adjusted for sex, and duration of diabetes. A P-value of <0.05 was considered statistically significant.

Results

Subject characteristics are shown in Table 1. Female participants were three times as many as their male counterparts. The majority (77.9%) of the subjects were >55 years old. The number of subjects with a duration of diabetes of <5 years, 5-10 years, and >10 years was nearly proportional (40.7% vs. 31.4% vs. 27.9%, respectively). According to BMI, most of the subjects fell into the overweight category (41.9%). Obesity was present in 20.9% of all participants. More than half (58.1%) of the subjects had an inflammation state, as indicated by hs-CRP values (≥3).

Table 1: Subject Characteristics (n=86).

Variable	n (%)
Age	
18-35 y	1 (1.2)
36-55 y	18 (20.9)
>55 y	67 (77.9)
Sex	
Male	23 (26.7)
Female	63 (73.3)
Duration of Diabetes	
<5y	35 (40.7)
5-10y	27 (31.4)
>10y	24 (27.9)
BMI	
Underweight	2 (2.3)
Normal	30 (34.9)
Overweight	36 (41.9)
Obesity	18 (20.9)
hs-CRP serum concentration	
<3 mg/L	36 (41.8)
≥3 mg/L	50 (58.1)

Table 2 shows the anthropometric measurements, muscle strength, and body composition in subjects classified as being in a non-inflammation and inflammation state. Patients in the inflammation state had higher body weight in corrected models (64.56 ± 11.19 vs. 59.67 ± 12.61; p2=0.02, p3=0.03). Using BMI as a comparison, the difference was significant across all models (27.44 ± 4.17 vs. 25.24 ± 4.21; p1=0.01, p2=0.02, p3=0.01). Among all the body circumference measurements, only neck circumference had no significant difference between the groups. No significant difference in the wrist circumference was observed, only in the unadjusted model. However, after adjustment for sex or sex and disease duration, the differences between groups were significant (p2=0.02, p3=0.01). Regarding

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Table 2: Comparison of anthropometric measurements between the group with and without inflammation (n=86).

Variables	hs-CRP serum concentration		p-value		
	<3 mg/L	≥3 mg/L	Model 1[b]	Model 2[c]	Model 3[d]
Weight (kg)	59.67 ± 12.61	64.56 ±11.19	0.06	0.02*	0.03*
Height (cm)	153.30 ± 8.06	153.3 1±7.27	0.99	0.51	0.73
BMI (kg/m2)	25.24 ± 4.21	27.44 ±4.17	0.01*	0.02*	0.01*
Waist circumference (cm)	86.53 ± 10.90	90.59 ±10.0	0.07	0.05	0.03*
Hip circumference (cm)	94.14 ± 12.40	99.43 ± 9.08	0.02*	0.02*	0.05*
Upper arm circumference (cm)	27.51 ± 3.89	29.51 ± 3.95	0.02*	0.02*	0.03*
Neck circumference (cm)	34.53 ± 4.19	35.38 ± 4.12	0.35	0.13	0.08
Wrist circumference (cm)	15.16 ± 1.22	15.67 ± 1.33	0.07	0.02*	0.01*
Handgrip strength (kg)	18.48 ± 14.19	19.79 ± 11.15	0.63	0.36	0.61
Triceps skinfold (cm)	19.22 ± 6.97	21.75 ± 8.50	0.14	0.18	0.17
Total body fat (%)	32.75 ± 6.44	35.81 ± 5.34	0.01*	0.01*	0.01*
Visceral fat (%)	10.44 ± 6.02	12.86 ± 6.18	0.07	0.03*	0.02*
Whole subcutaneous fat (%)	26.61 ± 7.47	29.92 ± 6.81	0.03*	0.01*	0.02*
Trunk subcutaneous fat (%)	23.91 ± 6.64	26.95 ± 6.14	0.03*	0.01*	0.01*
Arm subcutaneous fat (%)	39.84 ± 12.39	43.42 ± 11.29	0.16	0.09	0.12
Leg subcutaneous fat (%)	34.87 ± 9.39	39.16 ± 9.09	0.03*	0.01*	0.01*
Total muscle mass (%)	23.58 ± 3.42	22.78 ± 2.99	0.25	0.34	0.23
Trunk muscle mass (%)	17.31 ± 3.30	16.54 ± 2.65	0.23	0.32	0.25
Arm muscle mass (%)	26.81 ± 5.89	25.00 ± 5.63	0.15	0.12	0.14
Leg muscle mass (%)	37.48 ± 5.52	36.96 ± 4.62	0.64	0.94	0.98

Note: a unadjusted mean ± SD; b unadjusted p-value; c p-value adjusted for sex; d p-value adjusted for sex and duration of diabetes; * p<0.05

waist circumference, statistical significance was shown only after adjustment for sex and duration of diabetes (p3=0.03). No difference was found on triceps skinfold. In body composition, whole body, trunk, arm, and leg skeletal muscle showed no significant difference between groups. However, the group with higher hs-CRP showed overall higher body fat, except for the arm subcutaneous fat.

Several anthropometric measurements were correlated with the hs-CRP level, including body weight, BMI, waist circumference, hip circumference, and arm circumference. The associations remained significant even after adjustment for sex and duration of diabetes (Figure 1). Neck and wrist circumference only become significant after being adjusted. Arm muscle mass is the only muscle mass measurement that correlated with hs-CRP. All measures of percent body fat were correlated with hs-CRP (Figure 2).

Discussion

Being widely used for measurement of the nutritional status, BMI should be interpreted cautiously for its lack of accuracy to predict body fatness [26]. Several efforts have been made to create an alternative of BMI with comparable practicality yet perform better in predicting body fat mass. Waist circumference was shown to be a better predictor of adiposity [27] and mortality risk [28]. Recently studied in the United States, relative fat mass (RFM), calculated from height, waist circumference, and sex, predicted whole-body fat percentage

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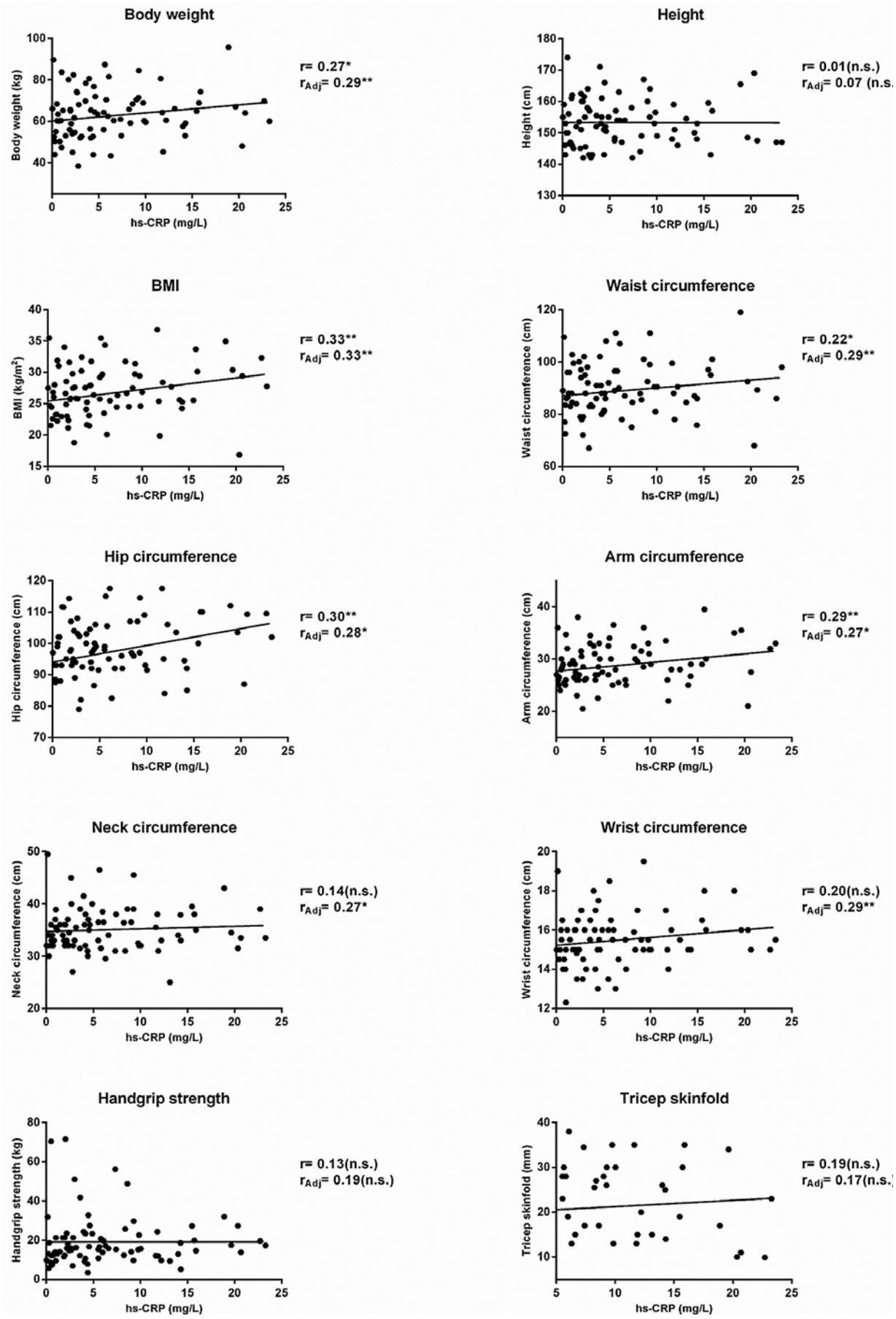

Figure 1: Correlation between anthropometric measurements and handgrip strength with hs-CRP concentration. r: Spearman's rank correlation coefficient, rAdj: correlation coefficient adjusted for sex and duration of diabetes, * $p<0.05$, ** $p<0.01$, (n.s.) no statistically significant.

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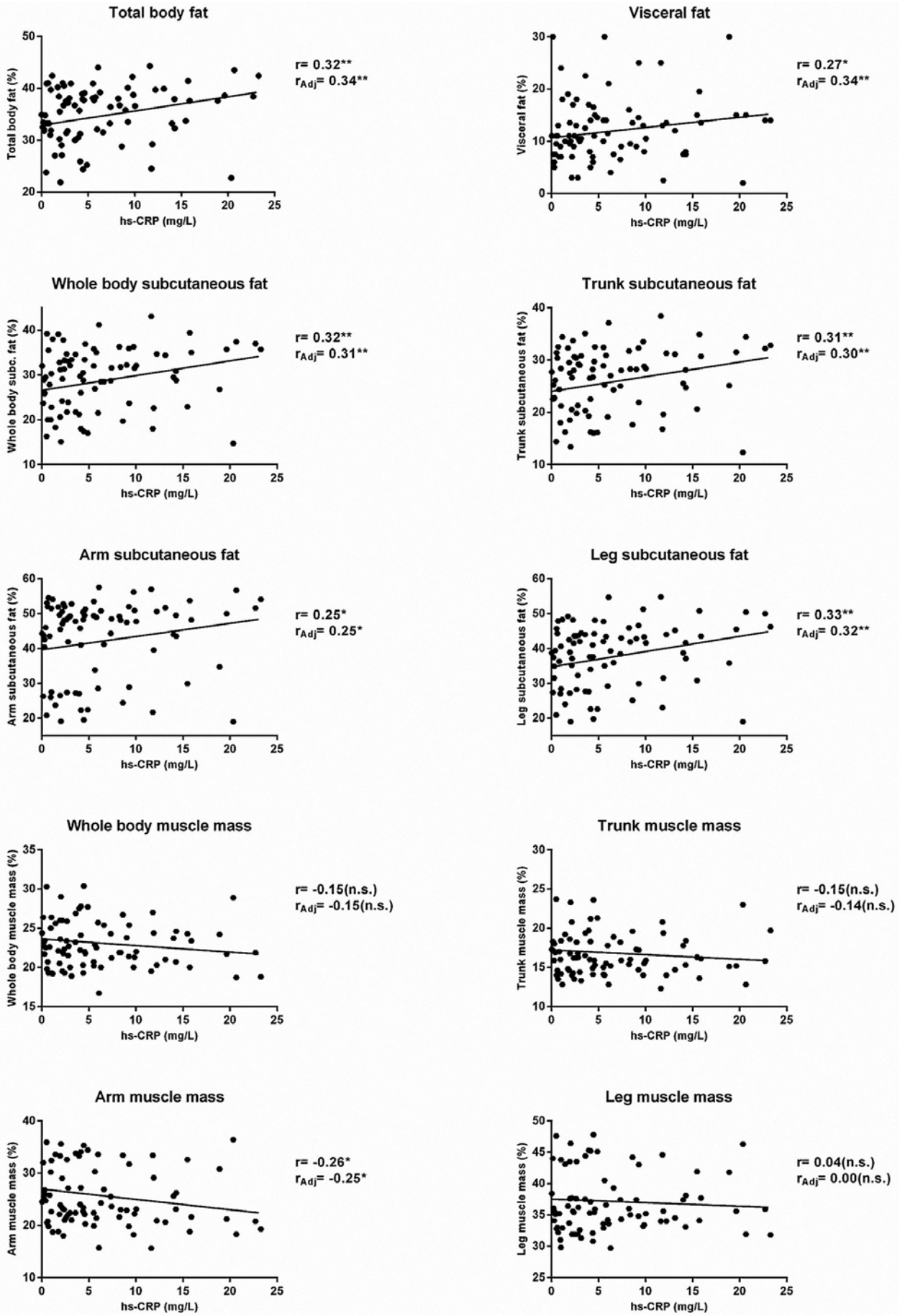

Figure 2: Correlation between body compositions with hs-CRP concentration. r: Spearman's rank correlation coefficient, rAdj: correlation coefficient adjusted for sex and duration of diabetes, * p<0.05, ** p<0.01, (n.s.) no statistically significant.

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better than BMI [29]. Among non-obese individuals, abdominal obesity is related to a high level of CRP independent of BMI [30]. However, the results of our study showed that the adjusted correlation of hs-CRP with BMI is similar to that of a more reliable inflammation predictor such as total, subcutaneous, and visceral body fat. This may indicate that for a population under investigation, BMI is as powerful as other better indicators in predicting inflammation.

Obesity is more prevalent in adults with T2DM compared with the general population. Data obtained from the 1999–2002 National Health and Nutrition Examination Survey (NHANES) indicated that in the United States, 85% of adults with T2DM patients are obese or overweight. Excluding the overweight, there are 54% obese adults among them [31]. In the same survey, the prevalence of adults with overweight and obesity in the US is 35% and 30%, respectively [32]. In Saudi Arabia, the prevalence of obesity in healthy adults and adults with diabetes was 12.05 and 20.68%, respectively [33]. It seems that the prevalence of obesity in people with diabetes was double compared to the general population. The increase of year by year diabetes prevalence was associated with the increase of obesity, not overweight prevalence [34].

Body fatness is associated with diabetes complications, which may be mediated by inflammatory processes [17]. Having similar BMI, diabetes was associated with nearly three times the risk of high percentage body fat (female: >28%; male: >20%) among Iraqi people [35]. This may partially explain why inflammatory markers such as hs-CRP, IL-6, and TNF-α tend to increase in people with diabetes [36]. Elevated pro-inflammatory cytokines levels are associated with diseases such as atherosclerosis [37], coronary heart disease [38], colorectal, and gastric cancers [39], and chronic renal diseases [40]. While inflammation is consistently associated positively with complications, the role of obesity in these diseases is more complicated. Acting as a risk factor for de novo kidney failure, obesity is associated with a lesser mortality rate in hemodialysis patients [41]. An inversed correlation between adiposity and the risk of retinopathy was observed [42, 43]. This suggests a paradoxical situation considering that inflammation, which is linked to body fatness, was positively associated with diabetic retinopathy [44].

Muscle mass was associated with a lower risk of developing T2DM [45]. Low muscle mass was found to increase the risk of all-cause mortality in the 11,687 participants of the 1999–2004 NHANES. Among people with obesity, those with low muscle mass have a 2.5 times higher risk of mortality than those with preserved muscle mass [46]. There is a high prevalence of low muscle mass among patients with T2DM. A Korean study conducted in newly diagnosed and drug-naive T2DM subjects showed that 12% had low muscle mass, and 8% had low muscle mass with abdominal obesity. Low muscle mass in this study was defined as appendicular skeletal muscle mass (ASM) ÷ height^2 of 2 SD below the mean value of the young reference group [47]. Among people with T2DM, low muscle mass is associated with insulin resistance [48, 49]. Low muscle mass was also associated with hyperglycemia in diabetes mellitus [50]. Inflammation contributes to muscle atrophy, possibly via impaired insulin signaling, which subsequently blunts Akt kinase activity [51]. It was observed that Akt activity in response to insulin was suppressed by 34% [52]. Reduced Akt activation results in phosphorylation of the FoxO transcription factors and transcription of MuRF1 and Atrogin-1, which result in muscle protein degradation [51]. In our study, inflammation is negatively correlated with arm muscle mass.

Measurements of wrist circumference were found to be correlated with inflammation in our study. Wrist circumference is used as a measurement to determine body frame size.

A recent study conducted in obese children suggested that wrist circumference is a biomarker of adipose tissue dysfunction and is a cardiovascular risk factor. Wrist circumference was shown to independently associated with the adiponectin-leptin ratio [53]. In a different study, wrist circumference was significantly correlated with metabolic syndrome by IDF (r=0.158), NCEPT-ATPIII (r=0.184), Cook's (r=0.197), and De Ferranti's (r=0.237) definition [54]. Most of the studies evaluating wrist circumference only involved children; therefore, more studies in the adult population should be conducted [55]. Among Ghanaian female patients with T2DM, wrist circumference was an independent predictor of metabolic syndrome [56]. The association between wrist circumference and the risk of cardiovascular disease may be related to anatomical anomalies in the heart. Wrist circumference is positively correlated with left ventricular dimensions and mass and epicardial adipose tissue in overweight children. The strongest association was found between wrist circumference and left ventricular end-diastolic dimension (r=0.73; p<0.0001). The association of left ventricular end-diastolic dimension with wrist circumference was markedly better than that with BMI (r=-0.04, p=NS) [57].

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Conclusion

We found that sex and duration of diabetes had a limited impact on the association between anthropometric measurements and inflammation.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Zhong J, Shi G. Editorial: Regulation of Inflammation in Chronic Disease. Front Immunol 10: 737, 2019
2. Yousuf O, Mohanty BD, Martin SS et al. High-Sensitivity C-Reactive Protein and Cardiovascular Disease: A Resolute Belief or an Elusive Link? J Am Coll Cardiol 62: 397–408, 2013
3. Li Y, Zhong X, Cheng G, et al. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: A meta-analysis. Atherosclerosis 259:7 5–82, 2017
4. Newcombe EA, Camats-Perna J, Silva ML, Valmas N, Huat TJ, Medeiros R. Inflammation: the link between comorbidities, genetics, and Alzheimer's disease. J. Neuroinflammation 15: 276, 2018
5. Wang J, Leung K-S, Chow SK-H, Cheung W-H. Inflammation and age-associated skeletal muscle deterioration (sarcopaenia). J Orthop Transl 10: 94–101, 2017
6. Wang X, Bao W, Liu J et al. Inflammatory Markers and Risk of Type 2 Diabetes: A systematic review and meta-analysis. Diabetes Care 36: 166–75, 2013
7. Calay ES, Hotamisligil GS. Turning off the inflammatory, but not the metabolic, flames. Nat Med 19: 265–7, 2013
8. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol 15: 505–22, 2018
9. Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE. Circulating Interleukin-6 in Relation to Adiposity, Insulin Action, and Insulin Secretion. Obes Res 9: 414–7, 2001
10. Sindhu S, Thomas R, Shihab P, Sriraman D, Behbehani K, Ahmad R. Obesity Is a Positive Modulator of IL-6R and IL-6 Expression in the Subcutaneous Adipose Tissue: Significance for Metabolic Inflammation. PLoS One 10: e0133494, 2015
11. Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: From physiopathology to therapy. J Hepatol 64: 1403–15, 2016
12. Chavakis T, Bierhaus A, Nawroth PP. RAGE (receptor for advanced glycation end products): a central player in the inflammatory response. Microbes Infect 6: 1219–25, 2004
13. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. Signal Transduct Target Ther 2: 17023, 2017
14. Senatus LM, Schmidt AM. The AGE-RAGE Axis: Implications for Age-Associated Arterial Diseases. Front Genet 8: 187, 2017
15. Kautzky-Willer A, Harreiter J, Pacini G. Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. Endocr Rev 37: 278–316, 2016
16. Peters SAE, Huxley RR, Woodward M. Sex differences in body anthropometry and composition in individuals with and without diabetes in the UK Biobank. BMJ Open 6: e010007, 2016
17. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci 13: 851–63, 2017
18. Tutuncu Y, Satman I, Celik S et al. A Comparison of hs-CRP Levels in New Diabetes Groups Diagnosed Based on FPG, 2-hPG, or HbAlc Criteria. J Diabetes Res 2016: 1–9, 2016
19. Wang Y, O'Neil A, Jiao Y et al. Sex differences in the association between diabetes and risk of cardiovascular disease, cancer, and all-cause and cause-specific mortality: a systematic review and meta-analysis of 5,162,654 participants. BMC Med 17: 136, 2019
20. Zoungas S, Woodward M, Li Q et al. Impact of age, age at diagnosis and duration of diabetes on the risk of macrovascular and microvascular complications and death in type 2 diabetes. Diabetologia 57(12): 2465-742014
21. Arambewela MH, Somasundaram NP, Ranjan Jayasekara HBP et al. Prevalence of Chronic Complications, Their Risk Factors, and the Cardiovascular Risk Factors among Patients with Type 2 Diabetes Attending the Diabetic Clinic at a Tertiary Care Hospital in Sri Lanka. J Diabetes Res 2018: 1–10, 2018
22. Østergaard L, Mogensen UM, Bundgaard JS et al. Duration and complications of diabetes mellitus and the associated risk of infective endocarditis. Int J Cardiol 278: 280–4, 2019
23. McCormack JP, Allan GM. Measuring hsCRP--an important part of a comprehensive risk profile or a clinically redundant practice? PLoS Med 7: e1000196, 2010
24. Sleman Health Office. Permudah Layanan Kesehatan Bagi Prolanis, Puskesmas Mlati II Luncurkan Inovasi Kretatif "Lapis Kenyal". Accessed at: https://dinkes.slemankab.go.id/permudah-layanan-kesehatan-bagi-prolanis-puskesmas-mlati-ii-luncurkan-inovasi-kretatif-lapis-kenyal.html 2019
25. CDC. Body Measurements (Anthropometry) - NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY III. Accessed at: https://wwwn.cdc.gov/nchs/data/nhanes3/manuals/anthro.pdf Rockville, MD: Westat, Inc.; 1988.
26. Rothman KJ. BMI-related errors in the measurement of obesity. Int J Obes 32(3): S56-9, 2008
27. Ashtary-Larky D, Daneghian S, Alipour M et al. Waist circumference to height ratio: Better correlation with fat mass than other anthropometric indices during dietary weight loss in different rates. Int J Endocrinol Metab 16(4), 2018

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28. Staiano AE, Reeder BA, Elliott S et al. Body mass index versus waist circumference as predictors of mortality in Canadian adults. Int J Obes 36(11): 1450-4, 2012
29. Woolcott OO, Bergman RN. Relative fat mass (RFM) as a new estimator of whole-body fat percentage - A cross-sectional study in American adult individuals. Sci Rep 8: 10980, 2018
30. Lapice E, Maione S, Patti L et al. Abdominal adiposity is associated with elevated C-reactive protein independent of BMI in healthy nonobese people. Diabetes Care 32(9): 1734-6, 2009
31. CDC. Prevalence of overweight and obesity among adults with diagnosed diabetes -United States, 1988-1994 and 1999-2002. Morb Mortal Wkly Rep 53(45): 1066-8, 2004
32. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. J Am Med Assoc 291(23): 2847-50, 2004
33. El-Hazmi MAF, Warsy AS. Prevalence of overweight and obesity in diabetic and non-diabetic Saudis. East Mediterr Heal J 6(2-3): 276-8, 2000
34. Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and Trends in Diabetes among Adults in the United States, 1988-2012. JAMA - J Am Med Assoc 314(10): 1021-9 2015
35. Mohammed SJ. Association between Percentage of Body Fat in Normal Body Mass Index Subjects and Type 2 Diabetes Mellitus in Iraqi Population: Case Control Study. J Diabetes Metab 8, 2017
36. Phosat C, Panprathip P, Chumpathat N et al. Elevated C-reactive protein, interleukin 6, tumor necrosis factor alpha and glycemic load associated with type 2 diabetes mellitus in rural Thais: A cross-sectional study. BMC Endocr Disord 17(1): 44, 2017
37. Adar SD, D'Souza J, Mendelsohn-Victor K et al. Markers of inflammation and coagulation after long-term exposure to coarse particulate matter: A cross-sectional analysis from the multi-ethnic study of atherosclerosis. Environ Health Perspect 123(6), 2015
38. Danesh J, Kaptoge S, Mann AG et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: Two new prospective studies and a systematic review. PLoS Med 5(4): e78, 2008
39. Taniguchi K, Karin M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. Semin Immunol 26(1): 54-74, 2014
40. Gluba A, Mikhailidis DP, Lip GYH, Hannam S, Rysz J, Banach M. Metabolic syndrome and renal disease. Int J Cardiol 164(2): 141-50, 2013
41. Rhee CM, Ahmadi SF, Kalantar-Zadeh K. The dual roles of obesity in chronic kidney disease: A review of the current literature. Curr Opin Nephrol Hypertens 25(3): 208-16, 2016
42. Chan JCY, Chee ML, Tan NYQ, Cheng CY, Wong TY, Sabanayagam C. Differential effect of body mass index on the incidence of diabetes and diabetic retinopathy in two Asian populations. Nutr Diabetes 8(1): 16, 2018
43. Man REK, Sabanayagam C, Chiang PPC et al. Differential association of generalized and abdominal obesity with diabetic retinopathy in Asian patients with type 2 diabetes. JAMA Ophthalmol 134(3): 251-7, 2016
44. Al-Kharashi AS. Role of oxidative stress, inflammation, hypoxia and angiogenesis in the development of diabetic retinopathy. Saudi J Ophthalmol 32(4): 318-323, 2018
45. Hong S, Chang Y, Jung H-S, Yun KE, Shin H, Ryu S. Relative muscle mass and the risk of incident type 2 diabetes: A cohort study. PLoS One 12: e0188650, 2017
46. Abramowitz MK, Hall CB, Amodu A, Sharma D, Androga L, Hawkins M. Muscle mass, BMI, and mortality among adults in the United States: A population-based cohort study. PLoS One 13(4): e0194697, 2018
47. Kim JA, Hwang SY, Chung HS et al. Proportion and Characteristics of the Subjects with Low Muscle Mass and Abdominal Obesity among the Newly Diagnosed and Drug-Naive Type 2 Diabetes Mellitus Patients. Diabetes Metab J 43(1): 105-113, 2018
48. Han SJ, Boyko EJ, Kim SK, Fujimoto WY, Kahn SE, Leonetti DL. Association of Thigh Muscle Mass with Insulin Resistance and Incident Type 2 Diabetes Mellitus in Japanese Americans. Diabetes Metab J 42(6): 488-495, 2018
49. Srikanthan P, Karlamangla AS. Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the third National Health and Nutrition Examination Survey. J Clin Endocrinol Metab 96: 2898–903, 2011
50. Sugimoto K, Tabara Y, Ikegami H et al. Hyperglycemia in nonobese patients with type 2 diabetes is associated with low muscle mass: The Multicenter Study for Clarifying Evidence for Sarcopenia in Patients with Diabetes Mellitus. J Diabetes Investig 10(6): 1471-1479, 2019
51. Perry BD, Caldow MK, Brennan-Speranza TC et al. Muscle atrophy in patients with Type 2 Diabetes Mellitus: Roles of inflammatory pathways, physical activity and exercise. Exerc Immunol Rev 22: 94-109, 2016
52. Krook A, Roth RA, Jiang XJ, Zierath JR, Wallberg-Henriksson H. Insulin-stimulated Akt kinase activity is reduced in skeletal muscle from NIDDM subjects. Diabetes 47(8): 1281-6, 1998
53. Luordi C, Maddaloni E, Bizzarri C et al. Wrist circumference is a biomarker of adipose tissue dysfunction and cardiovascular risk in children with obesity. J Endocrinol Invest 43(1): 101-107, 2019
54. Fazeli M, Mohammad-Zadeh M, Darroudi S et al. New anthropometric indices in the definition of metabolic syndrome in pediatrics. Diabetes Metab Syndr Clin Res Rev 13(3): 1779-1784, 2019
55. Namazi N, Djalalinia S, Mahdavi-Gorabi A et al. Association of wrist circumference with cardio-metabolic risk factors: a systematic review and meta-analysis. Eat Weight Disord 25(1): 151-161, 2018
56. Obirikorang C, Obirikorang Y, Acheampong E et al. Association of wrist circumference and waist-to-height ratio with cardiometabolic risk factors among type II diabetics in a Ghanaian population. J Diabetes Res, 2018
57. Zampetti S, Campagna G, Leto G et al. Relation Between Wrist Circumference and Left Ventricular Structure in Overweight Children. Am J Cardiol 121(12): 1624-1628, 2018

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Review

The Effects of Exenatide on Serum CRP Levels in Patients with Type 2 Diabetes: A Systematic Review of Randomized Controlled Trials

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Abstract

Introduction: There is a well-established link between type 2 diabetes and cardiovascular events risk. C-reactive protein is a biomarker of vascular inflammation used in the management of cardiovascular diseases. Exenatide, part of the glucagon-like peptide-1 receptor agonists, is used in type 2 diabetes treatment and has shown a decrease in cardiovascular risk factors. The objective of this systematic review is to evaluate the potential effect of exenatide on the C-reactive protein levels in patients with type 2 diabetes. **Material and Methods:** Medical databases, including PubMed®, Embase®, and Cochrane, were searched for randomized controlled trials based on the PICOS (P-Population, I-Interventions, C-Comparative interventions, O-Outcomes and S-Study design) framework. **Results:** 7 randomized controlled trials were included. Exenatide twice per day was used in all. Statistically significant decrease of serum C-reactive protein levels was reported in 4 randomized controlled trials in the exenatide twice per day group between baseline and end of the study. In 4 randomized controlled trials, exenatide twice per day decreased C-reactive protein levels more than metformin, sulfonylureas, and insulin glargine. **Conclusions:** Exenatide decreases serum C-reactive protein levels; therefore, it may decrease the inflammatory state associated with atherosclerosis, reducing the risk of cardiovascular diseases.

Keywords: Inflammation, Biomarkers, Glucagon-Like Peptide 1.

Introduction

Since 1980, the global age-standardized prevalence of diabetes in the adult population has nearly doubled, from 4.7% to 8.5% [1]. The most common type of diabetes is Type 2 Diabetes (T2D) and represents around 90% of all cases [2]. It is a heterogeneous disease characterized by high blood glucose because of a progressive loss of beta-cell insulin secretion often associated with insulin resistance [3].

In patients with T2D, treatment with glucagon-like peptide-1 receptor agonists (GLP-1RAs) improves glycemic control, as a result of multiple mechanisms including glucose-dependent insulinotropic and glucagonostatic effects, decreased food intake and delayed gastric emptying [4].

In 2005, the U.S. Food and Drug Administration (FDA) approved exenatide twice a day (EXBID). It was the first GLP-1RA used in the treatment of T2D. The exenatide once weekly (EXQW) was approved later

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and compared to EXBID demonstrated superior glycemic control, with fewer side effects, in particular, nausea [5].

Since 1979, from the Framingham cohort, a link between the presence of diabetes and subsequent cardiovascular events has been observed [6]. A meta-analysis conducted by The Emerging Risk Factors Collaboration highlighted that independently from other conventional risk factors, diabetes confers about a two-fold excess risk for a wide range of vascular diseases [7].

Vascular inflammation, associated with atherosclerosis, is one of the most common pathophysiological changes that characterize cardiovascular diseases (CVDs). To assist the detection of CVDs and to monitor their evolution, prognosis, and therapy implementation, inflammatory biomarkers are used. C-reactive protein (CRP) is an acute-phase protein whose serum concentration rises as a response of pro-inflammatory cytokines. CRP is a biomarker of the inflammatory process and has an essential role in atherosclerosis [8].

The long-term prospective cardiovascular outcomes trials (CVOTs) for new antidiabetic drugs were conducted after the release of FDA's recommendation for the pharmaceutical industry to demonstrate that treatment with antidiabetic therapies for T2D will not dramatically increase the cardiovascular risk [9]. As the results from the CVOTs for new antidiabetic drugs were published, several representatives of the GLP-1 RA group, besides demonstrating non-inferiority, highlighted the superiority in terms of their cardiovascular outcomes [10-14]. EXQW had CVOT with the highest number of patients. It proved, as intended, that the treatment with EXQW in patients with T2D with or without previous CVD does not significantly lead to differences between the incidence of major adverse cardiovascular events compared to placebo [15].

The aim of this systematic review of randomized controlled trials (RCTs) is to evaluate the potential effect of EXBID or EXQW on serum CRP levels in patients with T2D.

Material and Methods

We conducted a preliminary literature search, which included a broad approach to databases to find previously published reviews that analyzed the population and the targeted intervention.

We found systematic reviews and meta-analyses evaluating the same outcome, but the articles had been published more than 3 years ago and included, alongside EXBID or EXQW, other GLP-1 RAs. The current systematic review only assessed the effect of exenatide treatment in T2D patients on serum CRP concentration in RCT.

Previously published guidelines for systematic reviews were searched and used for writing this review [16, 17]. We based this review on a specific framework, PICOS; P (Population-patients diagnosed with T2D), I (Interventions-EXBID or EXQW), C (Comparative interventions-placebo or/and control group), O (Outcomes-CRP or hs-CRP) and S (Study design- RCT).

We included English written studies that were published from January 2005 until December 2019 inclusive. The studies were searched in the following databases: PubMed®, Embase®, MEDLINE®, Cochrane Database of Systematic Reviews (CDSR), Cochrane Central Register of Controlled Trials (CCTR) and https://www.clinicaltrials.gov for published and unpublished studies. The last search was on the 31st of January 2020.

For our search an association of relevant MeSH terms was used. In case of PubMed® we used ((“exenatide”[MeSH Terms] OR “exenatide”[All Fields]) AND (“diabetes mellitus, type 2”[MeSH Terms] OR “type 2 diabetes mellitus”[All Fields] OR “type 2 diabetes”[All Fields])) AND (“c-reactive protein”[MeSH Terms] OR (“c-reactive”[All Fields] AND “protein”[All Fields]) OR “c-reactive protein”[All Fields] OR “c reactive protein”[All Fields]) AND (Clinical Trial[ptyp] AND “humans”[MeSH Terms]).

Using the sites www.clinicaltrials.gov, or www.clinicalstudyresults.org we retrieved, if available and relevant, results of unpublished trials. Two reviewers (BA, AC) retrieved and screened the full text of the studies that meet the inclusion criteria.

The following exclusion criteria were: studies that did not have a randomized-controlled design, patients diagnosed with Type 1 Diabetes, patients without T2D, patients younger than 18 years, studies in which there was no sufficient information on CRP in every group in the beginning or at the follow-up (means and standard deviations (SD)).

Two researchers (BA and AC) assessed the risk of bias in the included RCTs following Cochrane criteria [18] independently. The following items were used for the assessment of each study: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, selection of the reported result, and other sources of bias [18]. Unclear issues were discussed with a third party (BC) and resolved by consensus.

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Results

In our search, we identified 127 records, as seen in Figure 1. After we checked for duplicates and excluded the studies that did not match the inclusion criteria, we analyzed seven RCTs.

Of the reviewed RCTs, three were multi-center, one included patients from 14 countries, one compared the EXBID treatment with a surgical procedure, and one included patients diagnosed with T2D and non-alcoholic fatty liver disease. EXBID was compared to placebo in 2 RCTs. The sample size ranged between 11 and

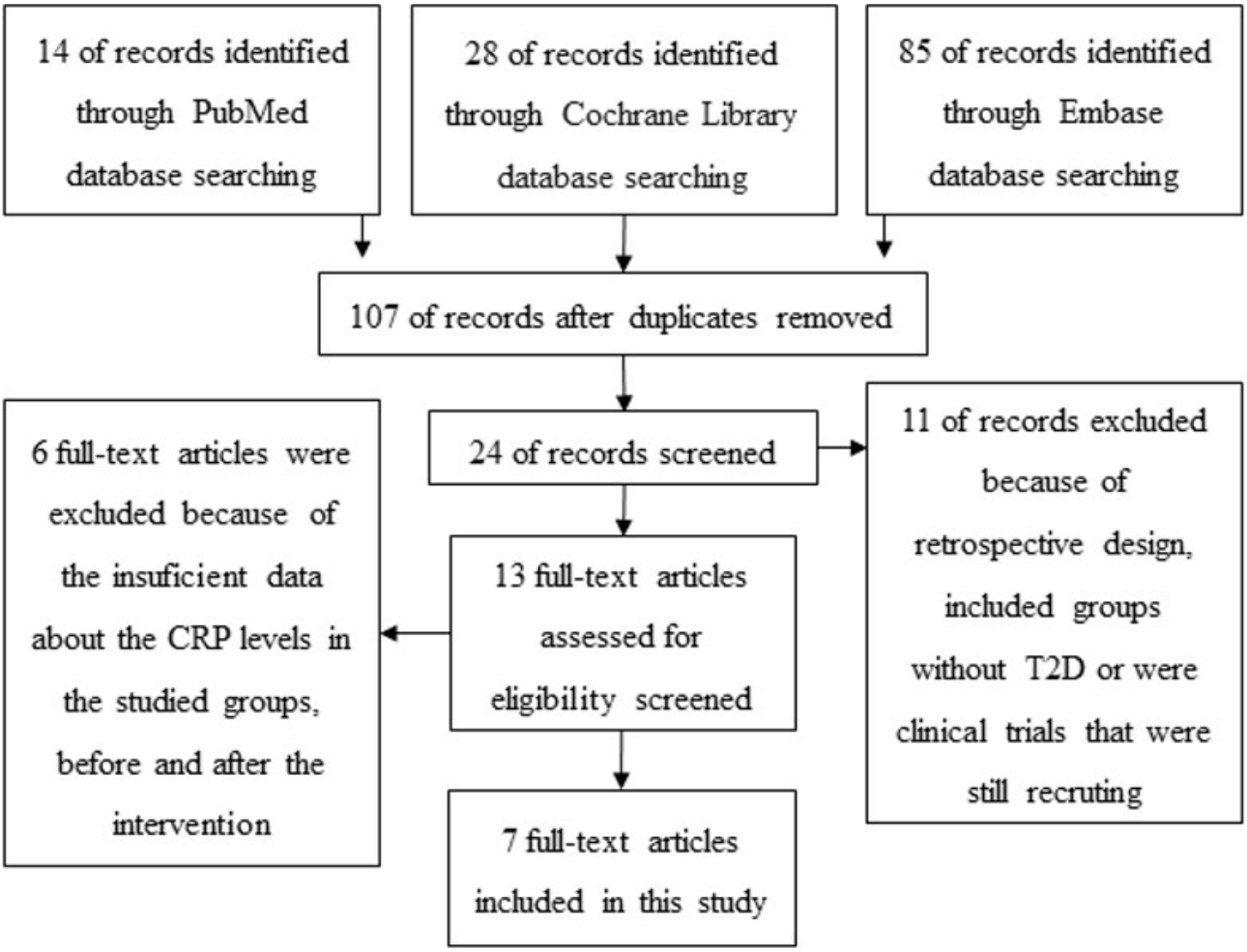
14 of records identified through PubMed database searching
28 of records identified through Cochrane Library database searching
85 of records identified through Embase database searching
107 of records after duplicates removed
24 of records screened
11 of records excluded because of retrospective design, included groups without T2D or were clinical trials that were still recruting
6 full-text articles were excluded because of the insuficient data about the CRP levels in the studied groups, before and after the intervention
13 full-text articles assessed for eligibility screened
7 full-text articles included in this study

Figure 1: Selection of the articles included in the study.

Table 1: Baseline characteristics of the included studies.

Study	Location	Duration of study	Study design	Number of participants (Male/Female)	Mean age of participants (years)	Mean duration of Diabetes
Derosa et al. [19]	Italy (Multi-center)	52 weeks	Randomized single-blinded controlled study	Exenatide 63 (30/33) Glibenclamide 65 (33/32)	Exenatide (57 ± 2) Glibenclamide (56 ± 6)	-
Wu et al. [20]	China (Nanjing)	16 weeks	Randomized double-blinded controlled study	Exenatide 12 (6/6) Placebo 11 (3/8)	Exenatide (57 ± 10) Placebo (54 ± 9.5)	Exenatide (7.3 ± 4.4) Placebo (5.0 ± 2.5)

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Derosa et al. [21]	Italy (Multi-center)	12 months	Randomized double-blinded controlled study	Exenatide 86 (43/43) Placebo 85 (41/44)	Exenatide (57.3 ± 7.7) Placebo (56.7 ± 7.3)	Exenatide (7.6 ± 2.8#) Placebo (7.8 ± 3.1#)
Fan et al. [22]	China (Beijing)	12 weeks	Randomized controlled study	Exenatide 49 (28/21) Metformin 68 (38/30)	Exenatide (51.02 ± 10.1) Metformin (54.68 ± 12.14)	-
Liang et al. [23]	China (Chonhquing)	12 months	Randomized controlled study	Exenatide 34 (24/10) RYGB 31 (22/9) Usual care 36 (24/12)	Exenatide (50.94 ± 5.89) RYGB (50.81 ± 5.44) Usual care (51.75 ± 6.70)	Exenatide (7.17 ± 1.80) RYGB (7.39 ± 1.69) Usual care (7.24 ± 1.61)
Gurkan et al. [24]	Turkey (Kocaeli)	26 weeks	Randomized controlled study	Exenatide 17 (5/12) Insulin glargine 17 (10/7)	Exenatide (52.18 ± 7.26) Insulin glargine (53.12 ± 6.99)	Exenatide (6.88 ± 3.23) Insulin glargine (7.59 ± 4.26)
Simo et al. [25]	14 countries (Multi-center)	36 months	Randomized controlled study	Exenatide 511 (56/44†) Glimepiride 508 (52/44†)	Exenatide (56 ± 10) Glimepiride (57 ± 9)	Exenatide (5.8 ± 4.8) Glimepiride (5.5 ± 4.3)

Note: s.d: #months; † percentage (%); RYGB=Laparoscopic Roux-en-Y Gastric Bypass.

Table 2: Pre- and post- treatment changes in hs-CRP in the included studies.

Study	Background therapy	Intervention	Baseline hs-CRP (mg/L)	End point hs-CRP (mg/L)
Derosa et al. [19]	Metformin, (before inclusion in the study) specific diet and physical exercise (after inclusion in the study)	Exenatide two times a day versus glibenclamide three times a day	Exenatide (2.1 ± 0.8) Glibenclamide (2.0 ±0.7)	Exenatide (1.5 ± 0.3**†) Glibenclamide (1.8 ±0.5)
Wu et al. [20]	Metformin and/or a sulfonylurea (before inclusion in the study)	Exenatide two times a day versus placebo	Exenatide (0.4 ± 0.5) Placebo (0.6 ± 0.4)	Exenatide (0.2 ± 0.3#) Placebo (1.4 ± 1.6)
Derosa et al. [21]	Metformin (given 8 ± 2 months before inclusion in the study), specific energy diet and physical exercise	Exenatide two times a day versus placebo	Exenatide (2.0 ± 0.8) Placebo (2.0 ± 0.8)	Exenatide (1.4 ± 0.2*) Placebo (1.6 ± 0.4)
Fan et al. [22]	-	Exenatide two times a day versus metformin two times a day	Exenatide (3.14 ± 0.58) Metformin (3.16 ± 0.68)	Exenatide (2.18 ± 0.34†) Metformin (2.69 ± 0.53)
Liang et al. [23]	Hypoglycemic medication, insulin (were continued during study for usual care group and exenatide + usual care group), diet and physical exercise. Pharmacological treatment was discontinued within 14 days after RYGB	Usual care and exenatide two times a day versus RYGB versus usual care	Exenatide (3.14 ± 1.14) RYGB (3.24 ± 0.95) Usual care (2.91 ± 1.55)	Exenatide (3.86 ± 0.57*) RYGB (1.52 ± 0.32*) Usual care (3.86 ± 1.34)

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Gurkan et al. [24]	Metformin (at least two months before inclusion in the study and maintained during intervention)	Exenatide two times a day versus insulin glargine once daily	Exenatide (0.87 ± 0.89) Insulin glargine (0.49 ± 0.42)	Exenatide (0.52 ± 0.47*) Insulin glargine (0.44 ± 0.47)
Simo et al. [25]	Metformin (before inclusion in the study and maintained during intervention)	Exenatide two times a day versus glimepiride two times a day	Exenatide (4.8 ± 7.7) Glimepiride (4.2 ± 5.1)	Exenatide (-1.74 ± 6.56††) Glimepiride (-0.2 ± 4.01)

Note: #p<0.05 compared to placebo; *p<0.05 compared to baseline; **p<0.01 compared to baseline;†p<0.05 compared to glibenclamide/metformin;††p<0.004 compared to glimepiride; RYGB=Laparoscopic Roux-en-Y Gastric Bypass; hs-CRP= high sensitive C-reactive protein.

511 patients. In two studies, we could not identify the diabetes duration. In one RCT, the gender distribution was presented as percentages. The studies varied in duration from 12 weeks to 36 months (Table 1).

We did not find RCTs that assessed the effects of treatment with EXQW on CRP and were eligible for inclusion.

CRP was significantly reduced in all studies, but in 4 RCTs, it was compared to baseline, in 3 RCTs was compared to the control group, and in 1 RCT was compared to placebo (Table 2).

There were some concerns regarding the risk of bias due to deviation from the intended interventions for 4 RCTs, but overall the risk-of-bias was low according to Revised Cochrane risk-of-bias tool for randomized trials (RoB 2).

Discussion

The findings in this systematic review are based on previous reports which highlighted the role of exenatide in decreasing serum CRP concentrations.

In the most recent RCT investigating the effect of EXBID versus Glimepiride in patients with T2D, serum hs-CRP levels were significantly reduced in the EXBID group (-1.74 ± 6.56 vs. -0.2 ± 4.01 mg/L, p=0.004) after 36 months of treatment [25].

EXBID was compared to another sulfonylurea, Glibenclamide, in a multi-centric RCT published by Derosa et al. in 2010. In this RCT, the decrease in hs-CRP after 52 weeks of treatment was statistically significant in the EXBID group (1.5 ± 0.3 mg/L) compared to the baseline value (1.9 ± 0.6 mg/L, p<0.001) and Glibenclamide group (1.8 ± 0.5 mg/L, p<0.05) [19]. The same author published in 2012 a multi-centric RCT in which the patients were given, after 8 ± 2 months of Metformin pre-treatment, EXBID, or placebo for 12 months. The difference in serum hs-CRP levels was statistically significant only in the EXBID + Metformin group after 12 months of treatment compared to baseline (2.0 ± 0.8 compared to 1.4 ± 0.2 mg/L, p<0.05). After 12 months of treatment, the differences between the EXBID + Metformin group and placebo + Metformin group were not statistically significant, nor were the differences between baseline and 12 months for the Placebo + Metformin group, concerning the serum hs-CRP levels [21].

Another EXBID vs. placebo RCT was published by Wu et al. in 2011. The study had the most reduced number of patients included in each group (12 treated with EXBID vs. 11 with placebo) and lasted for 16 weeks, compared to the other RCTs. The only statistically significant decrease of serum hs-CRP level was reported between the EXBID group and the Placebo group at 16 weeks of treatment (0.2 ± 0.3 vs. 1.4 ± 1.6 mg/L, p<0.05) [20].

Gurkan et al. reported a statistically significant decrease in serum hs-CRP only in the EXBID group between pre- and post-treatment parameters (0.87 ± 0.89 vs. 0.52 ± 0.47 mg/L, p<0.017), at 26 weeks of treatment. The comparator group was treated with Insulin glargine, and there was no statistically significant difference in the reduction of serum hs-CRP between groups or in the Insulin glargine group between baseline and week 26 of treatment [24].

In a 12 weeks study in which EXBID was compared to Metformin in patients diagnosed with concomitant T2D and non-alcoholic fatty liver disease, serum hs-CRP levels were markedly reduced in the EXBID group vs. Metformin group (2.18 ± 0.34 vs. 2.69 ± 0.53 mg/L, p<0.05) [22].

In another study, the EXBID group was compared to a usual care group and a surgical procedure group to assess the effect of laparoscopic Roux-en-Y gastric bypass surgery on T2D patients with hypertension. In the EXBID group, the reduction in serum hs-CRP levels af-

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ter 12 months of treatment was statistically significant compared with pre-treatment values (3.14 ± 1.14 vs. 3.86 ± 0.57 mg/L, $p<0.05$) [23].

We did not manage to include RCTs that evaluated the effect of EXQW treatment on CRP. During the screening phase of our study, we found papers that included groups of patients treated with EXQW, but the studies did not meet the inclusion criteria: the patients from the studies were not diagnosed with T2D [26-28]; the analysis was done retrospectively, on a subgroup of patients or the formulation of exenatide was not clearly stated [29-31].

The main limitation of our study was that we included only RCTs written in English, increasing the chance of selection bias. Another limitation was the heterogeneity of the trial periods, some being calculated in weeks [19, 20, 22, 24], other in months [21, 23, 25]. Also, the duration of diabetes before the inclusion in the trial was different, and several studies from this systematic review had small sample sizes [20, 24].

The potential benefit of exenatide on inflammatory markers that are linked to T2D is complex and needs further investigation.

Conclusion

Hs-CRP is an essential biomarker of inflammatory diseases such as CVD and T2D.

Our findings confirmed the association between exenatide and reduced serum CRP levels. Exenatide may, therefore, decrease the inflammatory state associated with atherosclerosis, reducing the risk of CVDs.

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Conflict of Interest

Cornelia Bala declared speaker and consultancy fees from: Astra Zeneca, Eli Lilly, NovoNordisk, Sanofi, Boehringer Ingelheim, Servier.

References

1. World Health Organization. Global Report on Diabetes. [online] Available at: https://apps.who.int/iris/bitstream/handle/10665/204871/9789241565257_eng.pdf?sequence=1&fbclid=IwAR2pWZUCkpOdXDgDDeUqE0KnJFzmn4oUiVG17GU75Q3Rh9wm_lHcyzFaaf0 [Accessed 10th Jan 2020]
2. International Diabetes Federation. Type 2 Diabetes. [online] Available at: https://idf.org/aboutdiabetes/type-2-diabetes.html?fbclid=IwAR1_7w-cuCKdLntyXicEaTjvi5X_3YFKY084WWgc0WHv0zjl4z0vSXckVME [Accessed 10th Jan 2020]
3. American Diabetes Association. Standards of Medical Care in Diabetes—2020. Diabetes Care 43(1):S1–S212, 2020 [online] Available at: https://care.diabetesjournals.org/content/43/Supplement_1/S1?fbclid=IwAR2CM5kZiU6UGLYt5Vdyy439-vGKT8jVQE4mX8CXuZBuEiPt9lAw34_vwuU [Accessed 10th Jan 2020]
4. Knop F, Brønden A, Vilsbøll T. Exenatide: pharmacokinetics, clinical use, and future directions. Expert Opin Pharmacother 18(6): 555-571, 2017
5. Blevins T, Pullman J, Malloy J et al. DURATION-5: exenatide once weekly resulted in greater improvements in glycemic control compared with exenatide twice daily in patients with type 2 diabetes. J Clin Endocrinol Metab 96(5): 1301–1310, 2011.
6. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. JAMA 241(19): 2035–2038, 1979.
7. Sarwar N, Gao P, Kondapally SR et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet 375(9733): 2215–2222, 2010.
8. Castro AR, Silva SO, Soares SC. The use of high sensitivity C-Reactive Protein in cardiovascular disease detection. J Pharm Pharm Sci 21: 496-503, 2018.
9. FDA. Guidance for Industry Diabetes Mellitus - Evaluating Cardiovascular Risk in New Antidiabetic Therapies to Treat Type 2 Diabetes [online]. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/diabetes-mellitus-evaluating-cardiovascular-risk-new-antidiabetic-therapies-treat-type-2-diabetes [Accessed 10th Jan 2020]
10. Sheahan KH, Wahlberg EA, Gilbert MP. An overview of GLP-1 agonists and recent cardiovascular outcomes trials. Postgrad Med J 96(1133): 156-161, 2020.
11. Marso SP, Daniels GH, Brown-Frandsen K et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 375(4): 311–322, 2016.
12. Marso SP, Bain SC, Consoli A et al. Semaglutide and cardiovascular outcomes in patients with Type 2 Diabetes. N Engl J Med 375(19): 1834-1844, 2016.
13. Hernandez AF, Green JB, Janmohamed S et al. Albiglutide and cardiovascular outcomes in patients with type 2 diabetes and cardiovascular disease (Harmony Outcomes): a double-blind, randomised placebo-controlled trial. Lancet 392(10157): 1519-1529, 2018.
14. Gerstein HC, Colhoun HM, Dagenais GR et al. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomized placebo-controlled trial. Lancet 394(10193): 121-130, 2019.

© 2020 The Authors

15. Holman RR, Bethel A, Mentz RJ et al. Effects of Once-Weekly Exenatide on Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 377: 1228-1239, 2017.
16. Liberati A, Altman DG, Tetzlaff J et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 6(7): e1000100, 2009.
17. Charrois TL. Systematic reviews: what do you need to know to get started? Can J Hosp Pharm 68(2): 144–148, 2015.
18. Higgins JPT, Savović J, Page MJ, Elbers RG, Sterne JAC. Chapter 8: Assessing risk of bias in a randomized trial. In: Cochrane Handbook for Systematic Reviews of Interventions. Higgins J, Thomas J, Chandler J et al. (eds). [online] Available at: www.training.cochrane.org/handbook [Accessed 15th Febr 2020].
19. Derosa G, Maffioli P, Salvadeo SA et al. Exenatide versus glibenclamide in patients with diabetes. Diabetes Technol Ther 12(3): 233–240, 2010.
20. Wu JD, Xu XH, Zhu J et al. Effect of exenatide on inflammatory and oxidative stress markers in patients with type 2 diabetes mellitus. Diabetes Technol Ther 13(2): 143–148, 2011.
21. Derosa G, Franzetti IG, Querci F et al. Exenatide plus metformin compared with metformin alone on β-cell function in patients with Type 2 diabetes. Diabetic Med 29(12): 1515-1523, 2012.
22. Fan H, Pan Q, Xu Y, Yang X. Exenatide improves type 2 diabetes concomitant with non-alcololic fatty liver disease. Arq Bras Endocrinol Metab 57(9): 702-708, 2013.
23. Liang Z, Wu Q, Chen B, Yu P et al. Effect of laparoscopic Roux-en-Y gastric bypass surgery on type 2 diabetes mellitus with hypertension: A randomized controlled trial. Diabetes Res Clin Pract 101: 50-56, 2013.
24. Gurkan E, Tarkun I, Sahin T, Cetinarslan B, Canturk Z. Evaluation of exenatide versus insulin glargine for the impact on endothelial functions and cardiovascular risk markers. Diabetes Res Clin Pract 106:567-575, 2014.
25. Simo R, Guerci B, Schernthaner G et al. Long-term changes in cardiovascular risk markers during administration of exenatide twice daily or glimepiride: results from the European exenatide study. Cardiovasc Diabetol 14: 116, 2015.
26. Lundkvist P, Pereira MJ, Katsogiannos P et al. Dapagliflozin once daily plus exenatide once weekly in obese adults without diabetes: Sustained reductions in body weight, glycaemia and blood pressure over 1 year. Diabetes Obes Metab 19: 1276-1288, 2017.
27. Kelly AS, Bergenstal RM, Gonzalez-Campoy JM, Katz H, Bank AJ. Effects of Exenatide vs. Metformin on endothelial function in obese parients with pre-diabetes: a randomized trial. Cardiovasc Diabetol 11: 64, 2012.
28. Wang X, Chen J, Li L et al. New association of bone morphogenetic protein 4 concentrations with fat distribution in obesity and Exenatide intervention on it. Lipids Health Dis 16: 70, 2017.
29. Bunck MC, Diamant M, Eliasson B et al. Exenatide affects circulating cardiovascular risk biomarkers independently of changes in body composition. Diabetes Care 33(8): 1734-1737, 2010.
30. Varanasi A, Chaudhuri A, Dhindsa S et al. Durability of effects of exenatide treatment on glycemic control, body weight, systolic blood pressure, C-reactive protein, and triglyceride concentrations. Endocr Pract 17(2): 192-200, 2011.
31. Chiquette E, Toth PP, Ramirez G, Cobble M, Chilton R. Treatment with exenatide once weekly or twice daily for 30 weeks is associated with changes in several cardiovascular risk markers. Vasc Health Risk Manag 8: 621-629, 2012.

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Review

Interaction of Adipokines with Diabetes Mellitus and Dental Implants

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Abstract

Diabetes mellitus is a chronic metabolic disease defined by the persistence of hyperglycemia leading to an inflammatory effect and a negative impact on bone formation and remodeling and impedes the osseointegration of dental implants. Dental implant surgery is a widely used procedure to replace missing teeth in patients who have diabetes. The success of an implant is dependent on osseointegration following placement. Adipokines are biologically active proteins secreted from adipose tissues, and they are considered to be involved in several functions like metabolism, immune response, inflammation. In the following article, we will describe the role of adipokines such as adiponectin, leptin, resistin, diabetes mellitus, and osseointegration of dental implants.

Keywords: Diabetes mellitus, leptin, adiponectin, resistin.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder marked by a defect in insulin secretion associated or not with peripheral insulin resistance, resulting in hyperglycemia. The different types of diabetes can be classified as immune-mediated (Type 1 Diabetes Mellitus – dependent only on insulin therapy), insulin resistance (Type 2 Diabetes Mellitus), gestational or other forms (genetic, infections, and due to some drugs). It is associated in particular with a higher or easier deterioration of the metabolism of carbohydrates, lipids, and proteins resulting from disorders in insulin secretion, its action in tissues, or both. This metabolic disorder is prevalent worldwide. According to the International Diabetes Federation, in 2017, 425 million people have been diagnosed with diabetes worldwide, representing about 8.8% of the adults, with equal rates in both women and men [1]. According to the World Health Organisation, by 2030, diabetes is going to be the 7th dominant cause of mortality [2]. The long-term complications associated with diabetes, if not controlled, comprise cardiovascular diseases, diabetic nephropathy, foot ulcers, diabetic retinopathy, and oral diseases. Oral complications are frequent, especially periodontitis, and lead to tooth loss, delayed wound healing, and impaired response to infection. Diabetes therapy is mainly aimed at keeping blood sugar levels within normal limits and can be accomplished with a balanced diet, exercise, weight loss, and use of medications (oral anti-diabetics or insulin therapy).

Diabetes mellitus and periodontitis have been biologically related. The ubiquity of periodontitis in diabetic patients is predicted to be double or triple as compared to non-diabetic patients; therefore, it is attributed as the sixth complication of diabetes [3]. Inadequate diabetes control results in rapid and severe periodontal tissue destruction due to increased oral microbial flora, leading to an excessive immune-inflammatory response to periodontal pathogens [4]. Dental implants can be an adjunctive treatment to missing teeth as a result of periodontal disease, an injury, tooth decay, or some other reason. The paucity of bone osseointegration and primary stability of the implant is contemplated to be

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the leading cause of implant failure. The constant hyperglycemia results in diminished bone formation, as it impedes osteoblastic activity and modifies the action of the parathyroid hormone, reduces collagen formation during callus formation, promotes apoptosis in lining cells of bone and boosts the osteoclastic activity [5]. As a result, bone formation is reduced. The benefits of dental implants appear to be satisfying in a diabetic patient with adequate treatment planning, prophylactic therapy, and appropriate postsurgical care only when glycemic levels are in normal limits.

The adipose tissue has significant capacity in energy storage and is also considered the largest endocrine organ that releases a number of biologically active proteins known as adipokines into the circulation. Adipokines function both locally and systematically and exhibit a range of receptors. Adipokines have a vital role in metabolism, different immune responses, and inflammation. In this review, we will describe the role of adipokines such as adiponectin, leptin, and resistin in diabetes mellitus and osseointegration of dental implants.

Leptin

Leptin is a non-glycosylated peptide hormone, synthesized and released from adipocytes. It has an impact on energy homeostasis, immune, and neuroendocrine functions [6] and was discovered in 1994 by Friedman and colleagues. In normal physiological conditions, leptin binds with specific receptors on appetite-modulating neurons and the arcuate nucleus in the hypothalamus. This leads to a decrease in appetite and to an increase in the energy expenditure, which, therefore, decreases adipose tissue mass and body weight, promotes glucose utilization, and ameliorates insulin sensitivity. The degree of adiposity can be observed by the level of leptin, and this is why it is also known as an “adipostat”.

Insulin secretion is inhibited by leptin in a negative feedback loop, whereas insulin provokes leptin secretion. Thus, impairment of the adipoinsular axis may increase the progression of insulin resistance. Leptin provokes fatty acid oxidation in the following way: it activates adenosine monophosphate-activated protein kinase (AMPK), particularly in muscle, resulting in a reduction of non-adipose tissue lipid stores [7]. Studies have shown higher leptin levels in persons with diabetes mellitus, probably reflecting leptin resistance. Leptin has the ability to improve or conversely induce insulin resistance as it mediates the release of insulin from pancreatic β cells. Martins et al. observed a significant correlation between leptin and obesity, hyperinsulinemia, and insulin resistance [8]. According to Piyali and colleagues, in agreement with Ko, the leptin/adiponectin ratio is a more accurate tool for insulin resistance than adiponectin or leptin levels alone [9].

Thomas et al. stated in 1999 that leptin could increase osteogenesis differentiation in mesenchymal stem cells from the bone marrow [10]. Bone osseointegration is an inflammatory and reparative process. Therefore, leptin levels are predicted to be higher during osseointegration. Leptin stimulates osteoblastogenesis and inhibits osteoclastogenesis through stimulation of osteoprotegerin and inhibition of receptor activator for nuclear factor-kB ligand (RANKL) expression in osteoblasts [11]. In 2010, Dilsiz et al. demonstrated an increase in leptin levels after 168 hours of orthodontic force application to teeth during orthodontic tooth movement [12]. Hassani et al., in 2017, stated the presence of leptin in the peri-implant sulcular fluid (PISF) and gingival crevicular fluid (GCF); but 60 days after the dental implant procedure, its levels did not show statistically significant changes [13]. Studies have submitted the positive effect of leptin on bone mineral density (BMD). Campos et al. described that increased levels of leptin: adiponectin ratio stimulate decreased BMD and bone mineral content (BMC) in obese adolescent girls [14].

Adiponectin

White adipose tissues synthesize and secrete a 30 kDa protein, known as adiponectin that circulates in high concentrations (5 - 30μg/ml) and represents about 0.01% of the total plasma proteins. Human adiponectin is found on chromosome 3q27. It is known to have anti-atherogenic and anti-inflammatory properties, and it also regulates insulin sensitivity. Adiponectin operates using two receptors located on skeletal muscles (Adipo R1) and liver (Adipo R2) [15]. Enhanced insulin sensitivity has been shown in both rodents and humans, when treated with thiazolidinediones, an anti-diabetic drug, as adiponectin is stimulated through peroxisome proliferator activating receptor - γ (PPAR-γ) agonists [16]. Several cross-sectional studies have documented that patients with obesity, insulin resistance, or Type 2 Diabetes Mellitus (T2DM) have low levels of plasma adiponectin [17, 18]. It was observed that females have statistically significantly increased values of serum adiponectin when compared with male’s counterpart, and this can be attributed to the distinctive body fat

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distribution and the response of sex hormones on the production of adiponectin, as androgens inhibit adiponectin secretion [19].

Adiponectin modulates insulin sensitivity via numerous metabolic processes. Globular adiponectin activates adenosine monophosphate-activated protein kinase (AMPK), mediated by Adipo R1, and peroxisome proliferator-activated receptor-α (PPAR-α), mediated by Adipo R2, thereby enhancing phosphorylation of acetyl-CoA carboxylase, fatty acid oxidation, glucose uptake. It also inhibits hepatic gluconeogenesis by impeding the hepatic enzyme phosphoenolpyruvate carboxylase, hence, decreasing tissue triglyceride amount in muscle and liver. Furthermore, adiponectin has an impact on the num¬ber of mitochondria and types of oxidative fibers [20]. Yamauchi et al. observed an absolute reversal of insulin resistance in lipoatrophic mice when treated with leptin and adiponectin together, in contrast to adiponectin or leptin alone [21].

Adiponectin improves bone formation and also inhibits bone resorption, as its receptors are expressed in osteoblastic and osteoclastic cells. In vitro studies have corroborated that adiponectin acts on osteoclasts to inhibit osteoclastogenesis and bone resorption and increases osteoblasts proliferation and differentiation. Adiponectin regulates bone turnover by stimulating the receptor activator of nuclear factor-κ B ligand (RANKL) expression and inhibiting the production of its decoy receptor, osteoprotegerin (OPG) [22]. The preliminary study performed by Bai et al. indicated that systemic administration of adiponectin (2.5 μg of mouse recombinant globular adiponectin per day) is expected to stimulate osteogenesis around peri-implant surface [23]. According to these observations, it could be proposed that adiponectin might lead to a significant decrease in the incidence of hyperglycemia and could enhance bone osseointegration in dental implant cases. Intake of soy protein, fish oils, and linoleic acid might lead to higher adiponectin levels [24].

Resistin

Resistin is an adipocyte-secreted polypeptide discovered in obese mice in 2001, and it has the capacity to interfere with the action of insulin, increasing insulin resistance in tissues. Human resistin is a cysteine-rich peptide of 12.5 kDa, with a sequence of 108 amino acids. In mice, resistin is secreted from white adipose tissue, while in humans, other than adipocytes, it is mainly secreted by peripheral blood mononuclear cells, TNF-α-induced macrophages, with the highest concentration in bone marrow cells. Resistin levels in human serum range from 7 to 22 μg/ml. Thiazolidinediones drugs down-regulates the expression of murine adipocytes through activating peroxisome proliferator-activated receptor – γ (PPAR-γ). Resistin has been associated with insulin resistance and inflammation. In rodents, resistin impairs glucose tolerance and insulin action, whereas, administration of an anti-resistin antibody improves glucose levels and insulin action [25].

Tokuyama et al. and Fujinami et al. illustrated significantly high resistin values in diabetic subjects compared to the control group [26, 27]. Al-Harithy and Al-Ghamdi also observed similar findings in obese diabetic subjects [28]. On the contrary, Heilbronn et al. and Takeishi et al. reported no significant changes in resistin concentrations among normal-weight subjects, obese non-diabetic subjects, and obese patients with diabetes mellitus [29, 30]. For these reasons, the role of resistin in human obesity is disputed. Chen et al. and Schwartz et al. interpreted that high serum resistin level subjects have a significantly higher risk of developing T2DM [31, 32].

Resistin stimulates osteoblastic and osteoclastic cells and enhances their differentiation. It influences the osteoclastic differentiation directly via activation of nuclear factor-kB promotor and proliferation of MC3T3-E1 cells in a protein kinase A (PKA)- and protein kinase C (PKC) dependent manner and expression of interleukin-6 in the cells. An increase in osteoblastic activity is indirectly through stimulation of interleukin-6 (IL-6) [33].

An inverse correlation is observed between resistin levels and osteocalcin levels in subjects having osteoporotic hip fractures [34]. The role and function of resistin in obesity, diabetes, and pathogenesis of osteoporosis it is still not certain, probably due to the limited studies existing until now.

Conclusion

Uncontrolled diabetes on dental implants can have an inflammatory effect and impedes the osseointegration of bone around implants. Adipokines play a major role in insulin sensitivity and inflammation. Also, adipokines can be useful in developing new medical strategies for prevention and treatment interventions in order to upgrade the success rate of dental implants in diabetic patients. However, further studies should be performed on the role of biomarkers in dental surgical therapy in the case of diabetic patients.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. International Diabetes Federation. IDF Diabetes Atlas, 8th edn. Brussels, Belgium: International Diabetes Federation. 2017.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 3(11): e442, 2006
3. Löe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care 16(1): 329–34, 1993
4. Nishimura F, Iwamoto Y, Soga Y. The periodontal host response with diabetes. Periodontol 2000. 43: 245-53, 2007
5. Kayal RA, Tsatsas D, Bauer MA et al. Diminished bone formation during diabetic fracture healing is related to the premature resorption of cartilages associated with increased osteoclast activity. J Bone Miner Res 22(4): 560-3, 2007
6. Al-Harithy, Rowyda N. Relationship of leptin concentration to gender, body mass index and age in Saudi adults. Saudi Med J 25(8): 1086-90, 2004
7. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Müller C, Carling D, Kahn BB. Leptin stimulates fatty acid oxidation by activating AMP-activated protein kinase. Nature 415(6869): 339-43, 2002
8. Martins MdC, Faleiro LL, Fonseca A. Relationship between leptin and body mass and metabolic syndrome in an adult population. Rev Port Cardiol 31(11): 711–19, 2012
9. Ko S-H. The adiponectin/leptin ratio and metabolic syndrome in healthy Korean adult males. Korean Diabetes J 34(4): 220–21, 2010
10. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinology 140(4): 1630–38, 1999
11. Karsenty G. Convergence between bone and energy homeostases: Review leptin regulation of bone mass. Cell Metab 4(5): 341-348, 2006
12. Dilsiz A, Kilic N, Aydin T, Ates FN, Zihni M, Bulut C. Leptin levels in gingival crevicular fluid during orthodontic tooth movement. The Angle Orthodontist 80(3): 504-8, 2010
13. Hassani A, Motamedi MHK, Bahar FM et al. Assessment of leptin concentration in peri-implant sulcular fluid during osseointegration of implants: Does it really vary? International Journal of Dentistry Research 2: 53-56, 2017
14. Campos RM, de Mello MT, Tock L et al. Interaction of bone mineral density, adipokines and hormones in obese adolescents girls submitted in an interdisciplinary therapy. J Pediatr Endocrinol Metab 26(3): 663-8, 2013
15. Spranger J, Kroke A, Möhlig M et al. F. Adiponectin and protection against type 2 diabetes mellitus. The Lancet 361(9353): 226-28, 2003
16. Astapova O, Leff T. Adiponectin and PPARγ: cooperative and interdependent actions of two key regulators of metabolism. Vitam Horm 90: 143-62, 2012
17. Kawano J Arora R. The role of adiponectin in obesity, diabetes, and cardiovascular disease. Journal of the CardioMetabolic Syndrome 4(1): 44-49, 2009
18. Ahsan S, Ahmed S, Ahmed S, Nauman K. Status of serum adiponectin related to insulin resistance in prediabetics. J Pak Med Assoc 64(2): 184-8, 2014
19. Bottner A, Kratzsch J, Muller G et al. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. J Clin Endocrinol Metab 89(8): 4053–61, 2004
20. Iwabu M, Yamauchi T, Okada-Iwabu M et al. Adiponectin and AdipoR1 regulate PGC-1α and mitochondria by Ca2+ and AMPK/SIRT1. Nature 464(7293): 1313–19, 2010
21. Yamauchi T, Kamon J, Waki H et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7(8): 941-6, 2001
22. Luo XH, Guo LJ, Xie H et al. Adiponectin stimulates RANKL and inhibits OPG expression in human osteoblasts through the MAPK signaling pathway. J Bone Miner Res 21(10): 1648–56, 2006
23. Bai Y, Yin G, Luo E. Adiponectin may improve osseointegration of dental implants in T2DM patients. Med Hypotheses 77: 192–94, 2011
24. Pischon, T, Girman CJ, Rifai N, Hotamisligil GS, Rimm EB. Association between dietary factors and plasma adiponectin concentrations in men. Am J Clin Nutr 81: 780–86, 2005
25. Patel L, Buckels AC, Kinghorn IJ et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 300: 472–76, 2003
26. Tokuyama Y, Osawa H, Ishizuka T et al. Serum resistin level is associated with insulin sensitivity in Japanese patients with type 2 diabetes mellitus. Metabolism 56: 693–98, 2007
27. Fujinami A, Obayashi H, Ohta K et al. Enzyme-linked immunosorbent assay for circulating human resistin: resistin concentrations in normal subjects and patients with type 2 diabetes. Clinica Chimica Acta 339(1-2): 57–63, 2004
28. Al-Harithy R, Al-Ghamdi S. Serum resistin, adiposity and insulin resistance in Saudi women with type 2 diabetes mellitus. Ann Saudi Med 25: 283–87, 2005
29. Heilbronn LK, Rood J, Janderova L. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. J Clin Endocr Metab 89: 1844-48, 2004
30. Takeishi Y, Niizeki T, Arimoto T et al. Serum resistin is associated with high risk in patients with congestive heart failure – A novel link between metabolic signals and heart failure. Circular 71: 460–64, 2007
31. Chen BH, Song Y, Ding EL et al. Circulating levels of resistin and risk of type 2 diabetes in men and women: results from two prospective cohorts. Diabetes Care 32: 329-34, 2009
32. Schwartz DR, Lazar MA. Human resistin: found in translation from mouse to man. Trends Endocrinol Metab 22: 259-65, 2011
33. Thommesen L, Stunes AK, Monjo M et al. Expression and regulation of resistin in osteoblasts and osteoclasts indicate a role in bone metabolism. J Cell Biochem 99(3): 824–34, 2006
34. Fisher A, Srikusalanukul W, Davis M et al. Interactions between serum adipokines and osteocalcin in older patients with hip fracture. Int J Endocrinol 2012: 684323, 2012

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Advertorial

PROGRESE ÎN TERAPIA INJECTABILĂ ÎN DIABETUL ZAHARAT TIP 2

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National Institute of Diabetes, Nutrition and Metabolic Diseases,"N. Paulescu"

Diabetul zaharat este un domeniu extrem de dinamic, cu modificări rapide de abordare terapeutică a ghidurilor internaționale, pe măsură ce apar noi cercetări, tehnologii sau terapii cu potențial de îmbunătățire semnificativă a stării de sănătate a persoanelor cu diabet zaharat. În acest scop, managementul diabetului zaharat tip 2 (DZ2) implică obligatoriu, dincolo de atingerea controlului glicemic propus, strategii multiple de reducere a riscului de complicații cronice, de morbi-mortalitate și de invaliditate.

Dintre noile clase terapeutice care și-au dovedit în ultimii ani eficiența, siguranța și tolerabilitatea în DZ2, se remarcă cea a agoniștilor de receptor GLP-1 (AR GLP-1), care prin dovezile științifice aduse, au contribuit la ultimele schimbări de paradigmă în terapia DZ2, AR GLP-1 fiind recomandați ca primă medicație injectabilă în DZ2 în detrimentul insulinei bazale, în marea majoritate a cazurilor de eșec al terapiei cu antidiabetice orale [1, 2]. La baza acestei recomandări a Consensului ADA-EASD se află efectul puternic antihiperglicemiant, însoțit de scădere ponderală, risc intrinsec redus de hipolicemie şi beneficiile cardiovasculare demonstrate pentru anumiți reprezentanți ai clasei AR GLP-1 [3].

Noii AR GLP-1 cu durată lungă de acțiune disponibili sunt caracterizați printr-o eficiență crescută asupra controlului glicemic și ponderal, fluctuații reduse ale concentrației plasmatice, tolerablitate gastrointestinală îmbunătățită, schemă de administrare mai convenbilă (o dată pe săptămână) și dispozitive de administrare care să faciliteze depășirea barierelor terapiei injectabile, implicit ducînd la amplificarea aderenței și persistenței terapeutice [4].

Unul din noii analogi de GLP-1 care întruneşte toate caracteristicile menționate mai sus, dulaglutid, a contribuit la ultima actualizare a Consensului ADA-EASD, prin includerea dovezilor rezultate din studiul său cu obiectiv cardiovascular, REWIND [5, 6]. Actualmente, dulaglutid este primul AR GLP-1 care a demonstrat protecție cardiovasculară la persoanele cu DZ2 și factori de risc, cu şi fără diagnostic anterior de boală cardiovasculară [6], spre deosebire de studiile precedente cu alți AR GLP-1, în care beneficiile cardiovasculare au fost validate doar pentru persoanele cu DZ2 şi cu evenimente cardiovasculare în antecedente [7, 8]. Prin eficacitatea sa antihiperlicemiantă susținută şi durabilă, însoțită de o scădere ponderală demonstrată în studii clinice randomizate, dar confirmată şi în studiile de viață reală, la care se adaugă beneficiile cardiovasculare demonstrate recent pentru o populație de pacienți cu DZ2 similară celei din ambulatorul de diabet [6, 9], dulaglutid se remarcă în terapia actuală a DZ2 ca o opțiune importantă pentru abordarea multifactorială a tratamentului DZ2.

Referințe: 1. Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, et al. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia* 61: 2461-2498, 2018; **2. Nauck MA, Meier JJ.** Management of endocrine disease: Are all GLP-1 agonists equal in the treatment of type 2 diabetes? *Eur J Endocrinol* 181: R211-R234, 2019; **3. Alexopoulos AS, Buse JB.** Initial injectable therapy in type 2 diabetes: Key considerations when choosing between glucagon-like peptide 1 receptor agonists and insulin. *Metabolism* 98: 104-111, 2019; **4. Gentilella R, Pechtner V, Corcos A, Consoli A.** Glucagon-like peptide-1 receptor agonists in type 2 diabetes treatment: are they all the same? *Diabetes Metab Res Rev* 35: e3070, 2019; **5. Buse JB, Wexler DJ, Tsapas A, Rossing P, Mingrone G, Mathieu C, et al.** 2019 Update to: Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 43: 487-493, 2020; **6. Gerstein HC, Colhoun HM, Dagenais GR, Diaz R, Lakshmanan M, Pais P, et al.** Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. *Lancet* 394: 121-130, 2019; **7. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, et al.** Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 375: 311-322, 2016; **8. Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jodar E, Leiter LA, et al.** Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 375: 1834-1844, 2016; **9. Scott LJ.** Dulaglutide: A Review in Type 2 Diabetes. *Drugs* 80: 197-208, 2020.

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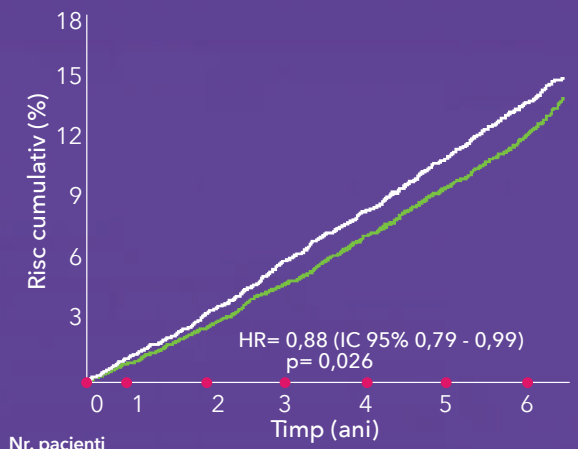
Obiectiv cardiovascular compozit (MACE3)



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MACE=Major Adverse Cardiovascular Events (evenimente adverse cardiovasculare majore)

Referințe: 1. Gerstein H.C et al; Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind,randomised placebo-controlled trial; Lancet. 2019; 394(10193):121-130; **2.** RCP Trulicity, octombrie 2019.

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- Pacienții cu diabet au risc de spitalizare cu **33%** mai mare din cauza **insuficienței cardiace** decât persoanele fără diabet.[5*]
- **Insuficiența cardiacă** este **motivul #1** de spitalizare a persoanelor cu vârsta peste 65 ani.[6]

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Referințe:

1. American Diabetes Association. *Diabetes Care*. 2019;42(suppl 1):S103-S112; **2.** Pecoits-Filho et al. Diabetol Metab Syndr. 2016;8(50); **3.** Alicic RZ et al. Clin J Am Soc Nephrol. 2017;12(12):2032-2045. **4.** Rosano GM, Vitale C, Seferovic P. Heart failure in patients with diabetes mellitus. Card Fail Rev. 2017;3(1):52-55. **5.** Cavender MA, Steg G, Smith SC et al. Impact of diabetes mellitus on hospitalization for heart failure, cardiovascular events, and death: outcomes at 4 years from the Reduction of Atherothrombosis for Continued Health (REACH) registry. Circulation. 2015;132:923-931. **6.** Azad N, Lemay G. Management of chronic heart failure in the older population. J Geriatr Cardiol 2014; 11(4):329–37. *Pe baza unei analize a datelor la 4 ani de la 45.227 de pacienți din registrul internațional REACH.[5]

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